THE ROLE OF COPEPODS IN THE DISTRIBUTION OF HYDROCARBONS: AN EXPERIMENTAL APPROACH

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ABSTRACT

Copepods may provide a significant pathway for the concentration and transfer of polyaromatic compounds (PAC) to higher trophic level consumers. PAC dissolved from weathered crude oil are more persistent in the environment and have much higher toxicity than the lighter, more volatile fractions of crude oil. Because of their polarity, PAC tend to accumulate in bio-lipids. Subarctic copepod species can contain up to 80% of their body dry weight in lipids and have a high surface area to volume ratio. Thus, PAC accumulation is rapid and bioaccumulation factors are in the order of 500 -8000, depending upon species and lipid content. While direct toxic effects of oil on copepods have been reported in the order of 10 mg/L, toxicity increases substantially in the presence of natural ultraviolet (UV) radiation. Phototoxic effects to the copepods *Calanus marshallae* and *Metridia okhotensis* were observed at concentrations of ~2µg/L total dissolved PAC followed by 4-8 hours of exposure to ambient daylight. Responses included mortality, immobilization and discoloration of lipid sacs. Further experiments were conducted to test the interaction effects of various concentrations of PAC dissolved from weathered Alaska North Slope crude oil and subsequent exposure to sunlight with and without the UVB component to the copepods *Neocalanus flemingeri* and *N. plumchrus*. Phototoxicity was found to be a linear function of the product of light intensity and PAC concentration. High natural variability in egg production rates precluded significant results of the toxicity of oil to copepod reproduction. This work has shown that copepods could potentially provide a mechanism for the concentration of dissolved PAC from the water and its transfer into pelagic and benthic food chains.

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OVERVIEW

The last 15 years have changed the understanding of oil toxicity mechanisms, in part achieved by numerous studies propagated by the *Exxon Valdez* oil spill in Prince William Sound in 1989. Among the significant advances are better understandings of the persistence and long term toxicity of larger polyaromatic compounds (PAC), which were previously thought to be of minor importance, compared to the lighter, more volatile but narcotic 1 and 2-ringed aromatic hydrocarbons. PAC were reported to cause genetic damage in fish, when early developmental stages were exposed to oil. Also, significant deposits of *Exxon Valdez* oil persisted on some beaches and continued to leak oil into the water for more than a decade after the spill. Concurrently with advances in oil toxicity studies, the relatively new field of phototoxicity received increasing scientific attention. The toxicity of specific polyaromatic hydrocarbons to various biota were found to increase up to 50 000 fold with UV radiation interaction. Phototoxicity of oil was also reported for several crude and fuel oils under light regimes likely encountered by biota in their natural environment. Chapter 1 is separated into three sections; the first two discuss the singular effects of UV radiation and oil on aquatic organisms, while the third section addresses advances in the field of phototoxicity.

PAC tend to accumulate in bio-lipids because of their polarity. As an abundant component of the pelagic community with a high surface area to volume ratio and a high bio-lipid content, copepods may provide a significant pathway for the concentration and transfer of PAC to higher trophic level consumers. *Neocalanus* copepods are the most abundant taxon of the zooplankton and can constitute over 60 % of the biomass during

the spring and summer months in the Gulf of Alaska and adjacent coastal regions. Many copepod species in polar and subpolar regions accumulate internal lipid stores of up to 60 to >80 % of their body dry weight. As an adaptation to the seasonally fluctuating supply of phytoplankton, which is their predominant food source, the late copepodite stages accumulate large lipid reserves during April to June in surface waters. Egg production and spawning can be delayed for several months and occurs at depth or during the spring ascent, timed to insure food abundance for the offspring during growth and fat storage. The potential of *Neocalanus* copepods for accumulation and transfer of PAC and a possible correlation to total lipid content was investigated (Chapter 2).

Accumulated PAC may act as internal photoreceptors, causing photo-oxidation in surrounding tissue. We studied the synergistic effect of exposure to dissolved PAC (~2µg/L) from Alaska North Slope crude oil and ultraviolet (UV) radiation in ambient daylight to the copepods *Calanus marshallae* and *Metridia okhotensis*. These were the first phototoxicity tests with translucent organisms that are at risk of exposure to dissolved PAC and UV radiation in Prince William Sound and the Gulf of Alaska. Responses included mortality, impairment of swimming ability and discoloration of lipid sacs. The interaction of the effect of PAC and UV radiation was highly significant ($P<0.005$) in two experiments (Chapter 3). Further experiments were conducted to test the interaction effects of various concentrations of PAC dissolved from weathered Alaska North Slope crude oil and subsequent exposure to sunlight with and without the UV-B component to the copepods *Neocalanus flemingeri* and *N. plumchrus* (Chapter 4). The results confirmed that phototoxicity is a linear function of the product of light intensity

and PAC concentration. The observed sensitivity of copepods to photoenhanced oil toxicity may have implications for the role of copepods in the transfer of hydrocarbons to other trophic levels: local populations could be subject to increased mortality if oil exposure is accompanied by or followed by sunny weather. This would cause food depletion for zooplanktonivorous fishes and may introduce PAC to the benthic food chain through sedimentation of dead copepods. The resulting reduction of energy flow from the primary production to higher trophic levels may have adverse effects on commercial fisheries.

An attempt was made to assess the toxicity of oil to copepod reproduction (Chapter 5). The feasibility of culture experiments to compare egg production rates and survival of oiled and unoiled female *Calanus marshallae* and *Pseudocalanus* spp. copepods was investigated. However, egg production rates varied greatly between females and between subsequent days. From these pilot experiments I concluded that the sample size needed to detect a significant difference between egg production of oiled and unoiled females was larger than could be obtained with the available methods.

While this research has demonstrated that copepods could potentially provide a mechanism for the concentration of dissolved PAC from the water and its transfer into pelagic and benthic food chains, the research does not attempt to assess the magnitude of this pathway. The retention time of oil in copepods and how dietary uptake of copepod-accumulated PAC affects predators remains to be investigated.

Chapter 1

THE SYNERGISTIC TOXICITY OF POLYAROMATIC HYDROCARBONS (PAH) FROM OIL AND ULTRAVIOLET (UV) RADIATION FROM SUNLIGHT TO AQUATIC SYSTEMS : A REVIEW

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ABSTRACT

Polyaromatic hydrocarbons (PAH) are common contaminants in aquatic systems, often introduced by human activities either as pulse perturbations (large oil spills) or press perturbations (chronic contamination at low levels). The toxicity of PAH to various biota is increased manifold by co-exposure to ultraviolet (UV) light. Significant advances of the past 15 years in the study of oil toxicity mechanisms and UV radiation impacts and the synergistic effects manifested in phototoxicity are summarized. This review focuses on research concerned with plankton and fish and those studies concerned with their physical environment. In addition, advances in explaining the chemical reactions involved in phototoxicity are included.

INTRODUCTION

The importance of investigating effects of photoenhanced toxicity was recognized in an early review of effects of near UV radiation (300-400 nm) on the toxicity of PAH in animals and plants (Arfsten et al., 1996). Included were studies on protozoans, cladocerans, insects, benthic invertebrates, aquatic vertebrates, plants and mammals. While many studies on terrestrial animals reported carcinogenic properties of PAH, which caused primarily skin damage, higher risks of phototoxicity in aquatic systems were suspected because of the immersion of the organisms in the dissolved PAH. In this review I focus on studies of aquatic organisms and populations, particularly zooplankton and fish, conducted in the past 15 years. It is not inclusive but summarizes major contributions to the understanding of phototoxicity of PAH in aquatic systems. Aquatic

organisms that are exposed to oil and sunlight experience not only the potential risk of phototoxicity but also the potential risk of toxicity of either of these factors alone. In order to address this point, the review is separated into three sections, of which the first two discuss the singular effects of UV and oil, while the third section addresses advances in the field of phototoxicity. First, the toxic effects of oil on aquatic organisms and their populations are summarized. This research, that was motivated by the *Exxon Valdez* oil spill (EVOS) in Prince William Sound, Alaska, has changed the perception of the relative toxicity of lighter and heavier aromatic compounds in oil. In addition, the scarce literature on oil effects on copepods is revisited. Secondly, the intensity and penetration of UV radiation in the water column and the effects of UV exposure to aquatic life forms are reviewed. It becomes evident that our understanding of the relation of spectral bandwidth and damage to organisms is still incomplete. Finally, an overview of advances in the field of phototoxicity since 1996 is presented.

The effects of oil pollution on aquatic populations

The reaction of the shoreline ecosystem to major hydrocarbon pollution was the focus of a recent review of studies motivated by the EVOS (Peterson 2001). Peterson reviewed acute, indirect and chronic effects, particularly those related to shoreline oiling and cleanup activities. Acute effects included the direct killing and injury of organisms along oil polluted coastlines that resulted in changes in species abundance and community structures. In the intertidal, blue mussels and five other bivalve species were identified as

ecological key species because: 1) particulate oil is concentrated during filter feeding, and, 2) their importance in the diets of many invertebrate and vertebrate species ("universal prey"). Mussel beds can retain and reintroduce largely unweathered oil to the surrounding environment for many years after the spill (Babcock et al., 1996; Carls et al., 2001). Another important factor affecting the recovery of inter-and subtidal ecosystems is the damage and removal of macroalgae and eelgrass. These plants provide shelter for a number of invertebrate species and their presence or absence changes the structure of the microhabitat. It is not surprising that a decline in algae cover and a decline in invertebrate abundance in the same areas occurred simultaneously. Peterson (2001) pointed out that an oil spill is both a pulse perturbation with subsequent recovery as well as a press perturbation with chronic and recurring contamination. Also, the shoreline habitat is an open system with effects being imported from and exported to adjacent systems. Secondary and lingering effects to fish, marine mammals and birds are difficult to assess. However, no evaluation of pelagic systems and the role of zooplankton was made (Peterson 2001).

Another review focused on studies related to impacts of the EVOS to pink salmon (Rice et al., 2001). The importance of secondary and lingering effects of oil in the environment was stressed, particularly where remnant oil pockets caused persistent chronic contamination. Results of conventional LC_{50} tests with juvenile fish indicated only mild acute toxicity of oil to these most sensitive life stages, but toxic effects of oil to aquatic populations were suggested to be largely underestimated because delayed effects were

not accounted for (Heintz et al., 2000). While conventional tests detected the toxicity of the lighter fractions of oil that readily evaporate (benzene, toluene, ethylbenzene and xylene (BTEX)), long term effects of the larger PAH were not detected. Pink salmon embryos that were incubated in more weathered oil during development had higher mortality than those incubated in less weathered oil (Heintz et al., 1999). This suggests that mortality was due to the toxicity of larger PAH rather than the narcosis mechanism that causes the toxicity of the lighter fractions of the oil. In Pacific herring, exposure of eggs to 0.7 ppb aqueous concentration of Prudhoe Bay crude oil increased occurrence of malformations, genetic damage, mortality, decreased size and inhibited swimming (Carls et al., 1999), possibly contributing to the population decline of Prince William Sound herring three years after the oil spill (Carls et al., 2002). As a result of these studies a revision of water quality standards for fish rearing habitats in Alaska from currently 15 ppb to 1 ppb was suggested (Rice et al., 2001).

Oil effects on zooplankton

Despite earlier research documenting that copepods accumulate hydrocarbons through the water soluble fraction of oil, oil droplets and through their diet, and may pass these accumulated hydrocarbons on to their predators (Corner 1975), effects of oil on zooplankton were not studied in the aftermath of the EVOS. Corner (1975) is also the only published source of data on depuration of oil by copepods over time. In an experiment with ^{14}C-labelled naphthalene, over 90 % of the radioactivity originally absorbed from solution by females of the estuarine species *Eurytemora affinis* was lost

after 6 days. Even so, this treatment with the hydrocarbon reduced lifespan and egg-production of the females by about 25 %. Further, when nauplii were treated in the same way, about 10% of the radioactivity originally present was still detected in the copepods a month later when they had become adults. Further experiments with *Calanus helgolandicus* studied dietary uptake of naphthalene by offering dead nauplii of *Elminius modestus* as prey. Thirty percent of the hydrocarbons were released within 24 hours, either unchanged or in the form of metabolites. However, all references are to unpublished experiments, so that an in depth review of procedures and data collection is not possible.

Experiments to compare changes in the plankton community of an untreated enclosure with those of an enclosure, to which No 2 fuel oil was added at a concentration of 50-60 ppb (measured by two methods) were conducted (Lee et al., 1977). The initial concentration declined rapidly over the course of the 19 day experiment. No major changes in the zooplankton standing stock were observed and a possibly reduced growth rate in *Pseudocalanus minutus* was attributed to the size shift from larger diatoms to smaller flagellates in the phytoplankton prey.

Possible exposure and uptake mechanisms of oil in zooplankton and effects on reproduction of copepods were reported (Capuzzo 1987). Acute toxicity of oil to copepods was reported at concentrations of 10 ppm (Spies 1987). However, the problem of assessing the impact of petroleum pollution on plankton in the field was considered

extremely difficult and impacts were believed to be minor and of short duration in comparison to those on benthos (Spies 1987).

The toxicity of ultraviolet light to aquatic organisms

Ozone depletion and UV-B

The observed depletion of stratospheric ozone and the subsequent increase in UV-B radiation at the earth's surface has propagated numerous studies on the toxic effects of UV-B exposure at increased levels (Smith and Baker, 1979). An overview of effects of these changes to aquatic systems is given by Worrest (1986) and an overview of studies concerning UV radiation in arctic ecosystems was recently published (Hessen 2002). Ozone depletion in the earth's stratosphere has manifested seasonal ozone holes over the Antarctic and the Arctic, causing increased UV-B radiation at the ocean's surface in these regions. The seasonal variations in ozone layer thickness are more pronounced at the poles than at lower latitudes (Frederick et al., 1989). The intensity of UV-B radiation at the earth's surface depends on absorption and scattering of solar radiation when it moves through the atmosphere but also on solar zenith angle, cloud cover and other particulate matter such as pollution in the atmosphere (Frederick et al., 1989). As a result of solar angle, the UV-B radiation at high latitudes is roughly half the maximum daily amount reaching temperate and tropical latitudes (Smith, 1989). Thus, the effective UV-B intensity may vary greatly depending on season, weather and geographical location.

UV penetration in water

UV-B radiation causes damage to fish larvae and juveniles, shrimp larvae, crab larvae, copepods and plants all of which are essential to the aquatic food web (Worrest 1986; Browman and Vetter, 2002). The extent of direct damage to aquatic organisms depends on the UV-B attenuating properties of the water and the UV avoidance or protection mechanisms of the organisms. Penetration depth of UV radiation in water is a function of dissolved organic carbon (DOC), humic substances, suspended particulates, algae and total chlorophyll content (Barron et al., 2000). In freshwater, UV penetration varies greatly between eutrophic and oligotrophic lakes (Williamson et al., 1994); (Zagarese et al., 1997).

Models were developed to assess the penetration of light, particularly UV-B, to depth in aquatic habitats. A model was developed to allow a quantitative calculation of the penetration of UV-B (280-340 nm) and of biologically effective dose-rates (to DNA) as a function of depth into various ocean water types (Smith and Baker, 1979). Several measurements of ocean water at different geographical locations were taken into account to derive attenuation coefficients. Further, UV-B penetration was calculated for three different ozone layer thicknesses showing differences in daily exposure time intervals, and intensity. The outcome of the model depends largely on the biological weighting function (i.e., what one defines as "biologically effective"),which considers the wavelength dependency of biological action (Smith and Baker, 1979). In a comparison with dosage-response results on anchovy larvae (Hunter et al., 1979), Setlows DNA

action spectrum (Setlow 1974) was chosen for the biological weighting function. A proportional increase of UV-B radiation at the surface resulted in an approximately similar proportional increase in UV radiation at all depths. Also, the effective DNA attenuation depth was approximately 6 m in the clearest ocean waters and about 2.5 m in moderately productive waters containing average amounts of dissolved organic matter.

Prediction of the effects of UV-B on aquatic populations has been difficult and estimated effects range from insignificant to detrimental (Smith 1989). Population effects depend on many physical and biological variables including, but not limited to, natural variability between taxa of aquatic organisms, their developmental stages and their reproduction rates, interaction with their physical and biological environment, behavioral responses and variations in effective UV-B radiation. In addition to the biological weighting function an amplification factor was recommended, which considers that a decrease in ozone layer thickness may have a much higher biological effectiveness (Smith 1989). Ozone decreases of 10 % resulted in an increase in effectiveness (DNA action spectrum) by 28 %. Ozone decreases by 20, 30 and 40 % are calculated to result in increases in biological effectiveness by 67, 125 and 213 %, respectively (Worrest 1986). Biological effectiveness also depends on mixing of the water column and the behavioral response to it. Mixing, or lack of mixing, can change the exposure of aquatic organisms to both UV-B and photosynthetically available radiation (PAR) by several orders of magnitude (Smith and Baker, 1979).

Solar spectral irradiance (SSI) in five different habitats, two marsh ponds, a shallow wetland, an estuary lagoon and the intertidal area of a high-energy sandy beach, was measured with either a scanning spectroradiometer (SSR) or a broadband radiometer (BBR) (Barron et al., 2000). Simultaneous measurements were taken at the surface of the estuary lagoon and used in instrument calibration of the BBR measurements. Water-quality parameters provided a general characterization of conditions but were not quantitatively associated with light attenuation. Because of wavelength-specific attenuation of solar spectral irradiance, ratios of visible light, UVA and UVB intensities will not be constant with changing water depth. Thus, habitat-specific measurements or estimates of SSI and UV-B are needed. At a depth of 1 m, SSI at the water surface can be reduced greater than 99%. But even at these levels of attenuation UV was sufficient to induce photoenhanced toxicity of a weathered oil to the tidewater silverside (Little et al., 2000).

Phytoplankton

Possibly the highest influence on global productivity would be caused by UV- induced growth reduction in marine phytoplankton. Exposure to UV-B at levels currently incident at the ocean's surface decreases algal productivity (Bothwell et al., 1994; Worrest 1986; Smith 1989). UV-B induced disruption of photosynthetic processes can include the electron transport system, photosystem-II reaction centers, pigment stability and DNA damage (Bothwell et al., 1994). For example, UV-B exposed cells of *Tetraselmis* sp. changed production of pigments (e.g., carotinoids) and fatty acids (Goes et al., 1994).

However, recovery from the stressful effects of UV-B occurred when the organisms were transferred back to control conditions. Despite inhibiting effects of UV-B on phytoplankton growth, ecosystem effects may be contrary, when higher UV sensitivity to the predator population reduces grazing pressure on the phytoplankton (Bothwell et al., 1994).

UV exposure experiments with Antarctic plankton were conducted in the austral spring (November-December) of 1987 (Bidigare 1989). Antarctic phytoplankton, like other phytoplankton found at lower latitudes, are susceptible to long-term exposure effects of UV radiation. Drastic changes in pigmentation were observed in seawater samples after exposure to ambient light levels, and a selective loss of chlorophyll-a was observed with increasing UV dose. In contrast to the results obtained with the 24 and 48 h experiments, short-term (4 h) exposure of ice algae to UV radiation produced no significant changes in the concentrations of chlorophyll-a, chlorophyll-c and fucoxanthin. However, these results were preliminary since accurate dosimetry with respect to the downward spectral irradiance of UV-B was missing. Despite these findings Antarctic phytoplankton probably receive extremely low UV doses during austral spring, due to their distribution (1) within or at the base of annual sea ice, (2) under the ice, and, (3) in poorly stratified water north of the annual sea ice (Bidigare 1989).

Zooplankton

Acute exposure of marine invertebrate zooplankton to UV radiation in the near-surface layer reduces survival and sublethal levels may reduce fecundity (Worrest 1986). In freshwater, solar UV radiation has long been suspected to influence the distribution of zooplankton (Zagarese et al., 1997). The vulnerability of three copepod species of the genus *Boeckella* to UV-B was compared (Zagarese et al., 1997). Three basic mechanisms to cope with harmful levels of UV-B radiation determined vulnerability: avoidance (e.g., vertical migration) and photoprotection (e.g., pigmentation) served to minimize exposure, while photorepair aided recovery from photoinduced damage. The three copepod species were collected from three lakes with differing depths and transparencies and subsequently incubated in the most transparent lake for 70 h at 50 cm depth. Each species had a dark control, a quartz treatment (full light spectrum) and a Mylar treatment (to exclude UV-B). Mortality in dark controls was low. Mortalities in the quartz and Mylar treatments were high for the transparent species *Boeckella gracilipes*, and lower for the pigmented species *B. gibbosa* and *B. brevicaudata*. Exposures to artificial UV-B radiation returned a similar pattern, showing that *B. gracilipes* was more than an order of magnitude more sensitive than *B. brevicaudata*. When exposed to artificial UV-B radiation in the presence of visible light, mortality of *B. gracilipes* was similar, suggesting little or no photorepair capacity in this species. For both other species mortality was lower in the presence of visible light than without.

Enclosure experiments with zooplankton communities at various exposure depths in an oligotrophic and an eutrophic lake were conducted under in situ light levels for 3 days (Williamson et al., 1994). No effect of UV-B radiation on the zooplankton community in the eutrophic lake at the shallowest depth (20 cm) was found. This corresponded with UV-B measurements, which had total attenuation of UV-B in the top 10 cm in this lake. However, in the oligotrophic lake UV-B related mortality and reduction of fecundity was observed for the copepod *Diaptomus* to the maximum tested depth of 6 m. Survival of two cladoceran species increased with depth in both the UV-B exposed and the UV-B shielded treatments, suggesting that longer wavelength radiation (UV-A) was a significant cause of mortality in these species. Survival was generally greater in the UV-B shielded treatment. Current UV-B levels may alter the vertical distribution and ecological interactions of some species in oligotrophic lakes, but may be a less important constraint in eutrophic lakes.

The effect of solar UV radiation on hatching of the marine copepod *Calanus finmarchicus* was studied in the Gulf of St. Lawrence, Canada (Alonso Rodigruez et al., 2000). In experiments under different light regimes hatching success was reduced in the presence of UV radiation at exposure levels that naturally occurred in the region at water depths where eggs hatch. Under natural sunlight UV-A appeared to be more detrimental to *C. finmarchicus* embryos than UV-B. While previous reports concentrated on the detrimental effects of UV-B and proved that phytoplankton was sensitive to these short and energetic wavelengths, the latter two studies with zooplankton found detrimental

effects in the UV-A wavelengths (Williamson et al., 1994; Alonso Rodigruez et al., 2000).

Fish

Few studies have been conducted on effects of ultraviolet radiation on fish. Northern anchovy (*Engraulis mordax*) and Pacific mackerel (*Scomber japonicus*) were compared with respect to the UV effects on eggs and larvae (Hunter et al., 1979). Eggs were exposed to different levels of UV light and egg mortality was monitored. Hatched larvae were measured and investigated with respect to UV induced abnormalities. Anchovy were more sensitive than Pacific mackerel. Irradiation induced lesions of the brain and eye caused marked dispersion of pigment within melanophores and retarded growth and development in surviving larvae. A decrease in ozone by 25 % would result in a significant increase in lesions and retardation of growth in anchovy at the surface, and to 3.5 meter depth at a 50 % ozone reduction (Hunter et al., 1979).

Atlantic cod eggs incubated at the surface and 50 cm depth had significant UV-B induced mortality (Beland et al., 1999). UV-A did not exacerbate or mitigate UV-B induced effects. No effects of UV-B exposure on the pigmentation of Atlantic cod larvae were observed. However, this result may have been due to small sample sizes, because only few larvae survived the experiments (Beland et al., 1999). Atlantic cod eggs in the first meter of the water column were susceptible to UV-B induced damage and mortality. Interestingly, either UV-A or UV-B alone was sufficient to cause a decline in vitality of

eggs of the copepod *Calanus finmarchicus*, while UV-A alone had no negative effect on cod eggs (Browman et al., 2000). On the other hand, UV-B produced a significantly greater negative effect on the cod eggs and mortality was strongly dose-dependent. A mathematical model that included the biological weighting functions, vertical mixing of eggs, meteorological and hydrographic conditions, and ozone depletion, indicated that UV-induced mortality in the *C. finmarchicus* egg population could be as high as 32.5 %, while the impact on the cod egg population was no more than 1.2 %. Despite these results UV effects in the natural environment might usually be overshadowed by other environmental factors, and only under a specific combination of conditions represent an important factor in controlling populations (Browman et al., 2000).

Photoenhanced toxicity of PAH

Phototoxicity mechanism

While the mechanisms involved in phototoxicity are still being studied, several studies have demonstrated photosensitization rather then photomodification to be the primary mechanism of phototoxicity to animals (Bowling et al., 1983; Allred and Giesy, 1985; Ankley et al., 1994; Pelletier et al., 1997; Barron et al., 2002). Photomodification is the light induced alteration of the dissolved chemical into a more toxic compound with subsequent uptake by the test organism. The postulated mechanism of photosensitization is absorption of a photoreceptive chemical into the organism which subsequently acts as a photoreceptor (Landrum et al., 1987; Small et al., 1967). Light energy excites the

photosensitizing chemical to a triplet energy state, which may then transfer energy to molecules within the cell or cell membrane, possibly generating reactive oxygen species (Landrum et al., 1987). Energy transfer can occur when the excited state energy of the photosensitizing chemical (e.g., PAH) exceeds that of an acceptor molecule such as oxygen which has a lower excited state energy (Zepp 1980). In particular, highly reactive singlet oxygen can be produced by spin-exchange with an excited triplet-state PAH (Turro 1987). The reactive oxygen species (e.g., singlet oxygen) can then cause tissue damage such as lipid peroxidation that would not be observed in the absence of UV (Livingstone 2001). An example for a reactive mechanism of photoenhanced toxicity involving lipid peroxidation was disruption of mucosal cell membrane function and integrity in fish gills (Weinstein et al., 1997). In contrast to the photosensitization mechanism, photomodification involves transformation of a chemical in the exposure water to a more toxic product, for example, photo-oxidation of phenanthrene to a hydroquinone. Studies demonstrating photomodification as the mechanism of photoenhanced toxicity of PAH have been limited to relatively high concentrations (2000 µg/L) of single compounds in the duckweed *Lemna gibba* (Huang et al., 1995) and phytoplankton (Marwood et al., 1999; Wiegman et al., 1999).

Identification of phototoxic PAH

A dose-response relationship for both anthracene concentration and solar radiation intensity in experiments with the cladoceran *Daphnia pulex* was discovered and photosensitization was identified as the mechanism of phototoxicity (Allred and Giesy,

1985). Chronic effects of photoenhanced toxicity of anthracene on *Daphnia magna* reproduction were reported (Holst and Giesy, 1989). Growing awareness of the problem of enhanced toxicity of chemicals including PAH under the influence of sunlight from these and other studies (e.g., Oris and Giesy, 1987) propagated the need to distinguish phototoxic from non-phototoxic PAH and establish parameters to aid in predictions about the phototoxicity of untested chemicals. A predictive structure-toxicity model was developed based on the phototoxicity of 20 PAH to *Daphnia magna* (Newsted and Giesy, 1987). In a curvilinear model, lowest triplet energy was an effective predictor for both LT_{50} and LC_{50} values adjusted to a constant PAH concentration in a parabolic relationship (Newsted and Giesy, 1987).

Another model to classify chemicals according to their phototoxic potential was based on calculated rather than measured structural properties (Mekenyan et al., 1994; Veith et al., 1995). The best descriptor to identify phototoxic from non-phototoxic chemicals was the energy difference between the highest occupied molecular orbital and the lowest unoccupied molecular orbital (HOMO-LUMO) gap. Aromatic chemicals that are phototoxic in sunlight have HOMO-LUMO gap energies between 6.7 to 7.5 eV. Generally, substituents had little influence on the phototoxicity of the parent compound. Recently, a different model improved the agreement of similar calculations with experimentally obtained singlet state energies over those results obtained from the HOMO-LUMO gap approach (Betowski et al., 2002).

The three-dimensional nature of the relationship between the tissue concentration of the phototoxic PAH, light intensity, and the exposure duration was investigated with the benthic oligochaet *Lumbriculus variegatus* (Ankley et al., 1995). Phototoxicity of sediment-associated PAH to these invertebrates had been demonstrated in a previous study (Ankley et al., 1994). The general applicability was supported for the Bunsen-Roscoe law, which states that in the absence of other "complicating" side reactions, the product of light intensity and reaction time is constant for a fixed concentration of the sensitizer (PAH) (Ankley et al., 1995). New water quality standards that take phototoxicity into account should consider that the local light regime is of equal importance as the concentration dependency of the phototoxic substance itself. Based on the theory that phototoxicity could only occur when the wavelengths present in the exposure overlap with the absorption spectrum of a specific PAH, three single compound PAH were tested with various light filters, which altered the UVA intensity but not the spectrum (Diamond et al., 2000). The results confirmed that differences between different light filters would be greatest in those PAH where differences between the filters affected the absorption spectrum most. This approach improved predictions of phototoxicity of specific compounds over calculations using total UVA intensity.

Phototoxicity of crude and fuel oils

All previous investigations focused on the phototoxic properties of single compounds and were conducted with freshwater species. The comparative toxicity of three single compounds (anthracene, flouranthene, pyrene), and four crude oils (Fuel Oil #2, Arabian

Light Crude, Prudhoe Bay Crude, Fuel Oil #6) to larvae and juveniles of the bivalve *Mulinia lateralis*, and juveniles of the mysid shrimp *Mysidopsis bahia* was investigated (Pelletier et al., 1997). Fresh crude oil -water mixtures were used. Phototoxicity occurred to marine species, and phototoxicity of petroleum products increased with their relative content of multiple aromatic ring structures (i.e., lighter crude oils were least phototoxic, heavier crude oils were more phototoxic).

Water collected from the Guadalupe oil field (California) was phototoxic to the mysid shrimp *Mysidopsis bahia* (Cleveland et al., 2000). An attempt was made to simulate field levels of sunlight exposure by first recording measurements of solar ultraviolet and visible light in the vicinity of the oil collection site, then using similar light exposure doses in the experiments. Mortality and growth reduction in the test organisms were observed at concentrations that were representative of expected concentrations of weathered oil and solar radiation at the study site. The phototoxicity of weathered oil might be higher than that of unweathered oil due to the larger relative amount of multiple aromatic ring structures.

The potential for photoenhanced toxicity in Prince William Sound, Alaska and the Gulf of Alaska was assessed (Barron and Ha'aihue, 2001). The light regime in the study area was evaluated: UV radiation was sufficient to cause photoenhanced toxicity at PAH concentrations that are likely after a spill event and may have been instrumental in resource damage observed in the aftermath of the EVOS. This resulted in an in-depth

study of possible sensitivity of eggs and larvae of Pacific herring to phototoxicity of aqueous phase and chemically-dispersed weathered Alaska North Slope crude oil (Barron et al., 2002). Toxicity increased with increasing concentrations of total PAH as well as solar intensity. While UV-A radiation alone caused significant phototoxicity, effects were greater if short exposures in natural sunlight were added. However, whether this was due to the UV-B component in sunlight or the higher UV wavelengths could not be discerned. Dispersants did not increase direct toxicity if equivalent total PAH concentrations in tissue were compared. However, bioaccumulation of oil into tissue of herring larvae was increased especially at the higher dispersant concentrations tested, causing direct toxicity of oil and phototoxicity to occur sooner than in oil-only exposures.

The observation of detrimental effects of low concentrations of PAH in the environment under the influence of sunlight has shifted research focus from large oil spill events (pulse perturbation) to recurring small scale contamination (press perturbation), especially in sunny, highly populated areas. Phototoxicity of PAH in freshwater lakes introduced by increased recreational use of two-stroke motorized watercraft was recently studied in Lake Tahoe, California/Nevada (Oris et al., 2002). Significant mortality in zooplankton (*Cerodaphnia dubia*) and 46 % reduction in growth of fish larvae (*Pimephales promelas*) were observed during two successive experiments in the summer of 1997. PAH concentrations in the lake were correlated with boating activity and phototoxicity as well as direct toxicity (reduced *Cerodaphnia* reproduction) were correlated with the concentration of contaminants. As a result, the Governing Board of

the Lake Tahoe Regional Planning Agency passed a ruling that banned the use of carburated 2-stroke engines from Lake Tahoe Basin effective June 1, 1999 (Oris et al., 2002).

CONCLUSION

Photoenhanced toxicity of oil is a function of numerous inter-playing variables: a) the composition of the oil; b) the specific spectral composition of sunlight and its energetic implications; and, c) PAH concentrations in tissue and light intensity. Each variable is subject to numerous influencing factors, which each in itself are subject to ongoing research efforts directed at understanding the mechanisms, which ultimately define the nature and degree of the observed toxic effects.

Traditional assays of the toxicity of oil were conducted with fresh oil, which in comparison to weathered oil has more lighter fractions (benzene, toluene, ethylbenzene, xylene). These lighter compounds are more volatile and cause higher immediate toxicity through a narcosis mechanism. In short-term toxicity tests the heavier fractions of the oil, which contain more aromatic ring-structures and are more persistent, caused little effect on the test organisms and were thus considered to be of minor toxicity. Within the last decade of studies on oil toxicity this view has changed: long term studies found that toxicity increased with the number of ring structures of the molecules; thus weathered oil with a higher proportion of multi-ring structures was more toxic than unweathered oil, with a lower proportion of the heavier compounds. The mechanism responsible for the

toxicity of multi-ring PAH was the destruction of DNA, and to a lesser degree, other biomolecules. At the same time, larger PAH were more persistent in the environment, leaching bioavailable contaminants years after an initial pulse perturbation.

In context with the study of photoenhanced toxicity, these persistent PAH had the structural properties to enable catalysis of tissue damage by absorption and transformation of solar energy. The advancing resolution of the structural properties of some known phototoxic PAH resulted in the publication of absorption spectra. These indicate not only that the inherent phototoxic potential of PAH depends on the molecular structure, but also that every molecule has a specific spectral region in which it can be activated. Thus, a photosensitizing molecule in an organism may be latent until the specific activating wavelength is encountered and initiates the phototoxic reaction.

The damaging influence of UV radiation, particularly the shorter wavelength UV-B radiation (280-340 nm), received considerable attention after the discovery of the decreasing trend of ozone content of the earth's stratosphere and the correlated increase in UV-B transmittance. Sensitivity to increased levels of UV-B radiation was found in virtually all biological systems, from primary producers to sensitive life stages of vertebrates and plants. Fundamental changes in productivity were predicted for some ecosystems, while others were believed to be relatively buffered from harmful UV-B penetration. However, phototoxicity has been observed with the exclusion of UV-B radiation, suggesting that the lower energy contained in longer wavelengths is sufficient

to catalyze the chemical reactions involved. Nevertheless, greater phototoxic effects of oil to fish were observed, when both UV-B and UV-A were used, indicating a possible inter-play of the different light spectra.

Phototoxic effects are a product of total PAH concentration in the test organism and exposure light intensity. In aquatic organisms with rapid molecular exchange over the outer surface or the gill tissue, accumulation of PAH is correlated with the total concentration of the chemical in the water. The sensitivity of biological organisms may depend on metabolic pathways that place photosensitive chemicals in more or less harmful positions. These sensitivities are apt to change over the course of a lifetime as detoxifying mechanisms develop (in fish and mammals) or chemical composition changes (e.g., increased fat storage in late stage copepods). The lipophilic nature of PAH favors tissues that are rich in bio-lipids for PAH deposition. Organisms with large fat deposits may be less apt to immediate damage than those which deposit the toxic compound in structural membranes, but accumulation potential may increase with fat content. Despite limited knowledge of the specific phototoxic compounds, there is a consistent trend of increasing phototoxicity with increasing total PAH concentration in test organism tissue, indicating that measurement of total PAH concentration is a good substitute for phototoxic compound.

The past 15 years of research in phototoxicity have promoted our understanding of the chemical processes involved and our ability to predict the phototoxicity of specific

compounds. Phototoxicity is of environmental concern in numerous aquatic systems at exposure levels present today. The first legal consequences of these findings were manifested in the ban of recreational 2-stroke carburated motorcraft from lake Tahoe. However, knowledge about ecosystem responses to increased PAH contamination or increased levels of UV radiation is limited and may be the central aspect of ecological studies related to phototoxicity in the coming decades.

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Chapter 2

BIOACCUMULATION OF POLYAROMATIC COMPOUNDS FROM OIL RELATIVE TO LIPID CONTENT IN THE COPEPODS *NEOCALANUS FLEMINGERI* AND *N. PLUMCHRUS*

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ABSTRACT

The bioaccumulation potentials of copepods for polyaromatic compounds (PAC) from aqueous solution in relation to total lipid content were investigated. *Neocalanus flemingeri* and *N. plumchrus* were sampled from four locations in Prince William Sound and the Gulf of Alaska between mid April and early June and experimentally exposed to low concentrations (0.5 - 12 µg total PAC/L) of dissolved Alaska North Slope crude oil. Total lipid content, lipid class composition and tissue accumulation of PAC was analyzed. Accumulation of PAC was passive and unselective. A positive correlation existed between total lipid content and bioaccumulation factors. In two samples with co-occurrence of the two *Neocalanus* species, no difference in lipid content or composition between species was found. *Neocalanus* copepods may aid in the concentration and transfer of PAC from the water column to higher trophic level consumers.

INTRODUCTION

The increased use of fossil fuels inevitably increases the risk of accidental spills of oil into the marine environment. Since many polyaromatic compounds (PAC) are known to have carcinogenic properties (Arfsten et al., 1996), the fate of PAC in the environment, sites of bioaccumulation, and identification of transport mechanisms are important. Bioaccumulation describes the augmentation of a substance in the tissue of an organism compared to its concentration in the surrounding environment. Bioaccumulation factors, as reported in this study, describe the ratio of PAC concentrations in copepod tissue to that of the exposure water. Also discussed is the potential role of copepods in transferring

accumulated PAC to higher trophic levels. Because of the polar attraction of oil-derived PAC to biogenic lipids, I hypothesized that copepods may accumulate dissolved PAC from the water and that the concentration factor is correlated to the total lipid content. While this correlation has been suspected (Corner 1975), chemical analysis of PAC concentrations in the exposure water, in tissue of exposed copepods and corresponding lipid class analysis has never been presented.

The congeneric and sympatric copepods *Neocalanus flemingeri* and *N. plumchrus* dominate the macrozooplankton biomass of the subarctic Pacific during spring and early summer (Miller 1988), and are an important component in the diet of many commercially important fish species (Cooney 1993). Perhaps as adaptations to the seasonality of high latitude systems, both species migrate vertically and accumulate large lipid stores during times of phytoplankton abundance in surface waters. Total lipid contents are among the highest measured in copepods and reach more than 80 % of dry weight (this study) with an average of > 60% during these months and consist predominantly of wax esters (Sargent and Henderson, 1986). These large wax ester stores are synthesized *de novo* by calanoid copepods, principally to fuel reproduction (Sargent and Henderson, 1986). Gonad development occurs in mid summer and fall, respectively, and spawning peaks in January for *N. flemingeri* and September for *N. plumchrus* at depths below 400 m (Miller and Clemons, 1988). Thus, large amounts of energy in the form of stored lipids in copepods are available to surface feeding predators during April to June, and this food

source then shifts to deeper layers of the water column for several months until lipids are diminished during gonad development and spawning (Sargent and Falk-Petersen, 1988).

Bioaccumulation of PAC by *Neocalanus flemingeri* was investigated experimentally. Copepods were sampled between mid April and the end of June, 2001 and at different locations in Prince William Sound (PWS) and the Gulf of Alaska (GOA) to obtain test organisms that would vary in lipid content. While *N. flemingeri* dominated the copepodite V size class in the spring, an approximately equal abundance of *N. flemingeri* and *N. plumchrus* occurred in early June, and a species comparison was conducted.

METHODS

Four separate experiments with identical experimental designs were conducted between mid April and early June of 2001 at the Institute of Marine Science in Seward, Alaska. Copepods were exposed to three levels of total PAC concentration in the exposure water (High, Low, Control) and then tested for PAC concentrations in their tissue. Each copepod sample consisted of n = 10 specimens, and 3 replicates were collected from each exposure dose. With each experiment three replicate samples of 10 copepods each were collected and analyzed for lipid content. For experiments 1 - 4 *Neocalanus flemingeri* copepodite stage V (CV) were used, experiment 4 was also conducted with *N. plumchrus* CV.

Sample collection

Zooplankton samples were collected with 200 µm mesh, open ring nets, equipped with altered design cod ends to minimize breakage of setae, and towed from 50m depth to the surface. All samples were collected in PWS and the Gulf of Alaska (Table 2.1) and kindly provided by researchers of the GLOBEC Gulf of Alaska Monitoring Program cruises, diluted if dense, and kept at ambient water surface temperatures until processed. In the laboratory, storage, sorting and experiments were conducted in a constant-temperature walk-in chamber at 6 - 8 °C. Copepods were pipetted into 1 ml culture wells for microscopic species and life stage identification and quickly transferred to beakers with 5 copepods per 50 ml beaker until the start of the experiment. Dry weight samples were immediately frozen and stored at -20 °C. The lipid samples from experiments 1 and 2 were initially frozen at -80 °C. Due to a temporary unavailability of the super cold freezer, storage of these samples and freezing of the samples for experiments 3 and 4 had to be moved to -20 °C for about 7 weeks, before all samples were again stored at -80 °C. Freezing caused little damage to the lipids but prolonged storage at -15°C did; rapid freezing followed by storage below - 70°C is recommended (Ohman, 1996).

Species identification

Neocalanus flemingeri and *N. plumchrus* were identified using a microscope according to visual criteria (Miller 1988): living CV of *N. flemingeri* bear patches of bright red while *N. plumchrus* has a more red-orange pigmentation. In addition, the pigmentation of the first antaennae in *N. flemingeri* is restricted to the base of the left antaenna, while *N.*

plumchrus bears pigment in both antaennae (Miller 1988). However, I observed that pigmentation was variable, especially between samples from different locations and diminished under stress. I observed many individuals with no coloration in the first antaennae. The size of the second maxilla was proportionately smaller in *N. flemingeri* than in *N. plumchrus* and was used as an additional clue. Specimens that did not fit the criteria for either species were not used. To verify species identification by these visual criteria, 8 randomly chosen copepods of each species were preserved and a scatter plot of cephalosome length against prosome length was plotted (see Figure 18, Miller 1988). From this I deduced that the error due to false species identification by visual criteria was < 15 %; small individuals of *N. plumchrus* can be mistaken for *N. flemingeri* if coloration is not distinct. In addition, a reference sample of 10 - 20 specimens was preserved at the time of sorting and 2-3 randomly selected copepods were dissected for microscopic inspection of the ventral tooth of the mandibular gnathobase, which bears 4 - 5 teeth in *N. flemingeri* while only 2 - 3 teeth are present in *N. plumchrus*.

Oil exposure and PAC analysis

The Alaska North Slope crude oil was weathered by heating and overnight stirring at 80 °C to 20 % weight loss, which removed most monocyclic aromatic compounds, then added to 2 and 3 mm diameter glass beads at application rates of 2.6 g oil/kg beads and tumbled for approximately 24 hours. The oiled beads were spread to single layer and left under a hood for 4 days at 25 °C to allow the oil to harden onto the beads, and then were stored at - 20 °C until use.

A detailed description of the generating columns that produced the aqueous solutions of PAC dissolved from crude oil is provided in Duesterloh et al. (2002). For the low dose treatments, one generating column was filled with 100 ml 3 mm diameter oil coated glass beads; for the high dose treatments 2 generating columns were filled with 100 ml of 2 mm diameter oil coated glass beads each and connected. In the control treatments the generating columns were filled with 100 ml of PAC-cleaned 3 mm diameter glass beads. Fresh columns were constructed for each experiment, except for experiment 3, which was conducted in close succession with experiment 2 with the same columns. Prior experience indicated that there was no loss in total PAC concentration from the columns within 96 hours.

Seawater was directed from the laboratory supply line into an overhead tank of approximately 80 liter capacity. It was then pumped through a generating column containing glass beads at a flow rate of 5 ml/min into a 2 liter Erlenmeyer filtration flask in which the hose connector served as an overflow. Each column was flushed with seawater for 22 hours before the peristaltic pump was activated and the flow rate in all columns was adjusted to 5 ml/minute. The experiment was started within 20 hours after activation of the pump, at which time 0.9 liter of the water in the Erlenmeyer flask was collected for PAC extraction and copepods were added to the remaining water volume in the flask. A small screen of 330 µm plankton mesh covered the outflow opening of the flasks to prevent loss of copepods. After 24 hours, copepods were collected and frozen

(-20 °C) and 0.9 liter of the exposure water were extracted with dichloromethane and then frozen for later PAC analysis at the Auke Bay Laboratory (NMFS/NOAA) in Juneau, Alaska.

Procedures for the quantitative determination of PAC in water and in tissues were described earlier (Short et al., 1996). Seawater samples (0.9 liter) were extracted twice with 50 - 60 ml dichloromethane. Copepod samples (n = 10) were macerated in a glass grinder twice, each time with 1 ml dichloromethane. Dichloromethane extracts of the PAC were reduced in volume and exchanged with hexane over a steam bath, followed by fractionation and purification by alumina/silica gel chromatography. PAC were measured by gas chromatography/mass spectrometry (GC/MS) in the selected ion monitoring mode (SIM). PAC analytes included dibenzothiophenes and polyaromatic hydrocarbons containing 2 - 5 rings, including the alkylated homologues listed in Table 2.2. A method blank, spiked method blank, and two reference samples were analyzed with each batch of 12 samples to verify method accuracy, precision, and absence of laboratory introduced artifacts and interferences. Detection limits were determined experimentally (Glaser et al., 1981) for PAC and generally were 5 - 20 ng PAC/L seawater at the 95 % confidence level. For tissues an 80 % confidence level was chosen. Concentrations below the detection limit were treated as 0.

To test whether there was a difference between the start and end total PAC concentrations in the exposure water, a paired comparison t-test was conducted. In this test the variation

introduced by possible differences between the experiments is eliminated by testing the mean difference and standard deviation of the difference in concentrations between start and end rather than by pooling of the means of all start and all end concentrations.

Lipid content and composition analysis

With each experiment, a corresponding sample of three replicates of *Neocalanus flemingeri* (n = 10) for lipid content and composition was measured. In experiments 3 and 4, lipid content and composition was also measured in *N. plumchrus*.

The lipid extraction method was modified from Christie (1982). The copepod sample was homogenized with 3 ml of 2:1 chloroform : methanol mixture. Twenty-five percent of the total volume of 0.88 % KCl in distilled water were added and after thorough mixing the top layer was discarded. After the addition of 25 % of the remaining volume of 1:1 distilled water : methanol and thorough shaking the mixture was allowed to separate. The purified lipid layer was volume reduced to 1 ml under nitrogen and 0.5 ml were dried and weighed. The remaining 0.5 ml were stored in a -20 °C freezer until analysis for lipid class composition with the High Pressure Lipid Chromatograph (HPLC) equipped with an Evaporative Light Scattering Detector (ELSD).

For the calculation of the total lipid percentage, mean dry weights were obtained from separate, corresponding samples (3 replicates, n = 10 copepods), which were thawed, weighed, and dried at 60 °C to constant weight (36 hrs). For experiments 1 and 2, dry

weight samples could not be obtained from the same live sample because of limited numbers of *N. flemingeri*. For experiment 1, I assumed that the dry weight was similar to that of copepods sampled in PWS two weeks later, and for experiment 2, dry weights from copepods sampled at the same time of the month in a different location in the Gulf of Alaska were used. For experiments 3 and 4 dry weights were measured from 3 replicate samples of n=10 copepods from the same live sample as the copepods used in the exposures.

Bioaccumulation Factors

Bioaccumulation factors (BAF) were calculated as follows (Barron 1994):

$$BAF = \frac{PAC_{Tissue}[ng/g] * 1000}{PAC_{Water}[ng/L]}$$

Note that in this equation, the tissue PAC concentration is weighted by the water PAC concentration. Tissue PAC concentrations are reported on a wet weight basis.

For each treatment, the BAF were regressed against the total lipid content of the corresponding copepod sample and the regression equation and correlation factors calculated (Figure 2.3).

RESULTS

PAC Exposures

The mean total PAC concentrations in the exposure seawater and their standard deviations (in parentheses) at the start and end of the 24 hr exposures (n = 4) were as follows:

TPAC concentration (µg/Liter)	start	end
High	10.98 (± 3.15)	11.05 (± 3.37)
Low	6.89 (± 1.95)	5.98 (± 1.05)
Control	1.25 (± 1.07)	0.72 (± 0.35)

The paired comparison t-test at the 95 % significance level resulted in no difference between start and end concentrations. Consequently, the mean value of start and end concentration was used in all subsequent calculations.

All control water samples had a distinctive PAC signature which was identified as typical for creosote contamination. Total PAC concentrations ranged from 0.42 to 2.84 µg/liter and averaged 0.91 µg/liter. However, concentrations of individual PAC were at least 10 times lower than experimental PAC concentrations (Figure 2.1). The background creosote signature in the test water originated most likely from old railroad ties and pilings which were lost into Resurrection Bay during the 1964 earthquake.

In copepod tissues, total PAC concentrations were approximately twice as high in the high dose compared to the low dose treatments. The variation between experiments was higher than in the water total PAC. As in the water samples, 3 - ring naphthalenes and monoaromatic pyrenes seemed to be accumulated in slightly higher proportions compared to the other analytes. Small amounts of C - 2 and C - 3 naphthalenes and acenaphthene were present in the tissue controls, but these were near the method detection limits of the individual analytes at the small sample weights used.

The patterns of concentrations of individual PAC analytes in the exposure water and the corresponding copepod samples are nearly identical, as would be expected for unselective and passive uptake (see for example Figure 2.1). A regression of total water PAC concentrations to total tissue PAC concentrations yielded a correlation coefficient (r) of 0.58 (Table 2.1). The proportions of individual analytes were consistent between high and low doses and between experiments with the possible exception of 3-ring naphthalenes and mono-aromatic pyrenes, which seemed to be present in slightly higher proportion in the high dose compared to the low dose.

Lipid content and composition

There was no consistent trend of increasing lipid content with sampling date. Lipid contents obtained with the gravimetric method ranged between 17 and 88% of copepod dry weight (Table 2.1).

There was no difference in total lipid content [%] between co-occurring *N. flemingeri* and *N. plumchrus*:

	GOA, May 16	Cape Cleare, June 1
N. flemingeri	84.10	41.01
N. plumchrus	84.64	40.21

On average, lipids were composed of 84 ± 10.3 % wax esters, 8 ± 7 % cholesterol, 9 ± 5 % free fatty acids and no triacylglycerol (mean ± 1 standard deviation)(Figure 2.2). The difference in lipid composition between *N. flemingeri* (N = 15, sampled at 5 locations, dates) and *N. plumchrus* (N = 6, sampled at two locations, dates) was significant for the wax ester/cholesterol ester group and the cholesterols, but it was not significant, when the Cape Cleare samples were excluded from the test. Copepods of both species from the Cape Cleare sampling location had a significantly higher free fatty acid component than those from the other sampling locations (t-test, $\alpha = 0.05$). If copepods from the same samples were compared, there was no difference between species.

Statistical analysis

The regression equations of bioaccumulation factors (BAF) and total lipid content had a positive slope for the high and low dose treatments (Figure 2.3). These slopes were significantly different from zero (P = 0.59) in the high dose treatment but not significantly different from zero in the low dose treatment. In the control treatment, the correlation coefficient was low (r = 0.08), compared to r = 0.33 in the high and r = 0.46 in

the low dose treatments. Plots of jackknife residuals against the predicted value showed no violations of regression assumptions in any of the regressions.

DISCUSSION

Bioaccumulation of dissolved PAC from the surrounding water by copepods was correlated to total lipid content. However, the positive linear relationship was significant only in the high but not in the low dose treatment. The correlation coefficients were 0.33 and 0.46 for the high dose and low dose treatments, respectively, and reflect the large spread of the data around the regression line. In contrast, there was a poor correlation ($r = 0.08$) and a slightly negative slope of the regression line in the control treatment. The similar slopes in the low and high dose regressions indicated that uptake occurred below saturation concentrations: BAF are expected to be constant at lower concentrations and decrease when saturation levels or lethal doses are approached. Lethal doses of oil to copepods were reported at seawater concentrations of 5 - 10 mg PAC/L (Spies 1987). In comparison, PAC exposure concentrations in this study were lower by a factor of 1000. The formula for BAF considers both the exposure water concentration and the accumulated tissue concentration of PAC. BAF were not significantly different (t-test, alpha = 0.05) between the low and high dose treatments, while a significant correlation existed between water and tissue PAC concentrations. Consequently, higher tissue concentrations in copepods can be expected when PAC exposure concentration, lipid content, or both increase.

The spread in the data is largely a result of variation in copepod weights. Both the weights and the lipid contents varied greatly between sampling dates and locations. An assumed increase in lipid content of copepods between April and June was not supported by data. However, Hopcroft (University of Alaska Fairbanks, unpublished data) observed that over all life stages, *N. flemingeri* sampled in PWS in May were consistently heavier than those sampled in April. While the assumption of seasonally increasing total lipid content may hold true for a local population, differences between sampling locations in the study region were more pronounced. For example, Cape Cleare copepods were smaller (mean prosoma length 4.16 mm, N = 10) and only 20% of the dry weight of PWS copepods (mean prosoma length 5.01 mm, N = 10). The great variation in weights and lengths was also reflected in datasets collected independently by researchers of the GLOBEC program (Chris Stark, University of Alaska Fairbanks, unpublished data). High interannual variation in dry weights and also in lipid free dry weights of *N. flemingeri* in the GOA and a rapid increase in stored lipid between May 8 and May 18, 1988 were reported earlier (Miller, 1993).

The agreement in total lipid content and composition between co-occurring copepods of the two *Neocalanus* species in this study indicates no energetic difference. In the assessment of oil spill effects on copepods and the role of copepods in the possible transfer of oil to higher trophic levels, the two species could be treated as one. However, I caution against the general conclusion of equal lipid content and composition of the two species, because my conclusion is based only on two sampling locations and differences

may exist in other geographical regions. *Neocalanus* abundance shifted from exclusively *N. flemingeri* in spring to co-occurrance of *N. flemingeri* and *N. plumchrus* in mid May and early June. This was in accordance with the reported life history analysis (Miller and Clemons, 1988). Total lipid content in *N. flemingeri* during May was measured and values ranged from 12 - 44 % of dry weight (Miller 1993). While the low value was attributed to an early developmental stage, the high values were believed to be low compared to other years and calanoid copepods in general (Miller 1993). In comparison, total lipid content of *N. flemingeri* in this study ranged from 17 - 88 %. However, in the calculation of lipid content, a higher copepod dry weight results in a lower estimate of total lipid content. If copepod weight increased in the two weeks between collection of the lipid and dry weight samples for experiment 1(see methods), the calculated low value of 17 % lipid may be slightly underestimating the true lipid content of this sample. Similarly, it is possible that the high value of 88 % is a slight overestimation. However, two additional samples of *N. flemingeri* and *N. plumchrus*, obtained in the GOA during May were in the 80 - 85 % range. Lipid class composition of co-occurring *N. flemingeri* and *N. plumchrus* in this study was not significantly different. However, the ratio of wax esters to free fatty acids and tri-acyl-glycerides changes during gonad development and egg production (Sargent and Falk-Petersen, 1988). Consequently, because of the differently phased life histories of the two species, a difference in lipid class composition might be expected during other times of the year.

Neocalanus copepods may provide an important mechanism for the transfer of dissolved PAC from oil to higher trophic level consumers like fish. This research has demonstrated that *Neocalanus*, due to their large lipid stores, aid in the concentration of dissolved oil from the water. Early research has identified copepods as relatively insensitive to oil compared with other plankton organisms (Capuzzo 1987). This insensitivity might be explained by the association of the oil derived PAC to the wax esters in the lipid stores of the copepods, which remain metabolically inactive until onset of gonad development and egg production. When freshly oiled plankton was fed to pink salmon, negative effects on growth and survival of the fishes were observed (Carls et al., 1996). The retention time of passively accumulated oil from aqueous solution in copepods remains to be investigated.

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Figure captions

Fig. 2.1: Concentrations of PAC analytes in exposure seawater (N=2) and copepod tissue (N=3) on a dry weight basis in experiment 2. Grey bars indicate concentrations in the High and Low dose treatments; black bars are concentrations in the respective controls. Categories on the X-axes are the PAC analytes listed in Table 2.2 in sequential order from left to right.

Fig. 2.2: Proportions of the major lipid classes in *Neocalanus flemingeri* (N = 15) and *N. plumchrus* (N = 6). Bars depict standard deviations. See text for discussion of the significance of the difference between species. WE/CE = Wax Esters, Cholesterol Esters; TAG = Tri-acyl-glycerides; CHO = Cholesterol; MON = Mono-acyl-glycerides; FFA - Free fatty acids, PE = Phosphatidyl-ethanolamine; PC = Phosphatidyl-choline.

Fig. 2.3: Correlation between bio-accumulation factors (BAF) and total lipid content in *Neocalanus* copepods in High dose, low dose and no oil (control) treatments. Circled data points are *N. plumchrus*., uncircled data points *N. flemingeri*.

Table 2.1: Summary of analytical data and derived bio-accumulation factors (BAF). N.f. = *Neocalanus flemingeri*; N.p. = *N. plumchrus*; dw = dry weight; *Dry weight derived from different live sample than test organisms (see text for further explanation).

Experiment	1	2	3	4	4
Date	041601	050301	051401	060101	060101
Species	N. f.	N. f.	N. f.	N. f.	N. p.
Location	PWS	GOA	Cape Cleare	GAK1	GAK1
dry/ind (mg)	0.716*	0.392	0.124*	0.317	0.313
% lipid (dw)	17	88	80	41	40
Water TPAC (ng/L)					
High	15384	10223	10861	7587	7587
Low	8376	6214	6215	4950	4950
Control	2024	797	625	495	495
Tissue TPAC (ng/g)					
High	12729	19618	12505	13391	15090
Low	7317	11071	5834	7952	4152
Control	616	368	642	553	951
BAF					
High	827	1919	1151	1765	1989
Low	874	1782	939	1607	839
Control	304	462	1028	1117	1922

Table 2.2: Polyaromatic compounds measured in exposure water and copepod tissue.

1) naphthalene
2) 1-methylnaphthalene + 2-methylnaphthalene
3) C-2 naphthalenes
4) C-3 naphthalenes
5) C-4 naphthalenes
6) biphenyl
7) acenaphthylene
8) acenaphthene
9) fluorene
10) C-1 fluorenes
11) C-2 fluorenes
12) C-3 fluorenes
13) dibenzothiophene
14) C-1 dibenzothiophenes
15) C-2 dibenzothiophenes
16) C-3 dibenzothiophenes
17) phenanthrene
18) C-1 phenanthrenes/anthracenes
19) C-2 phenanthrenes/anthracenes
20) C-3 phenanthrenes/anthracenes
21) C-4 phenanthrenes/anthracenes
22) anthracene
23) fluoranthene
24) pyrene
25) C-1 fluoranthenes/pyrenes
26) benz-a-anthracene
27) chrysene
28) C-1 chrysenes
29) C-2 chrysenes
30) C-3 chrysenes
31) C-4 chrysenes
32) benzo-b-fluoranthene
33) benzo-k-fluoranthene
34) benzo-e-pyrene
35) benzo-a-pyrene
36) perylene
37) indeno-1,2,3-cd-pyrene
38) dibenzo-a,h-anthracene
39) benzo-g,h,i-perylene

Figure 2.1: Concentrations of PAC analytes in exposure seawater and copepod tissue on a dry weight basis.

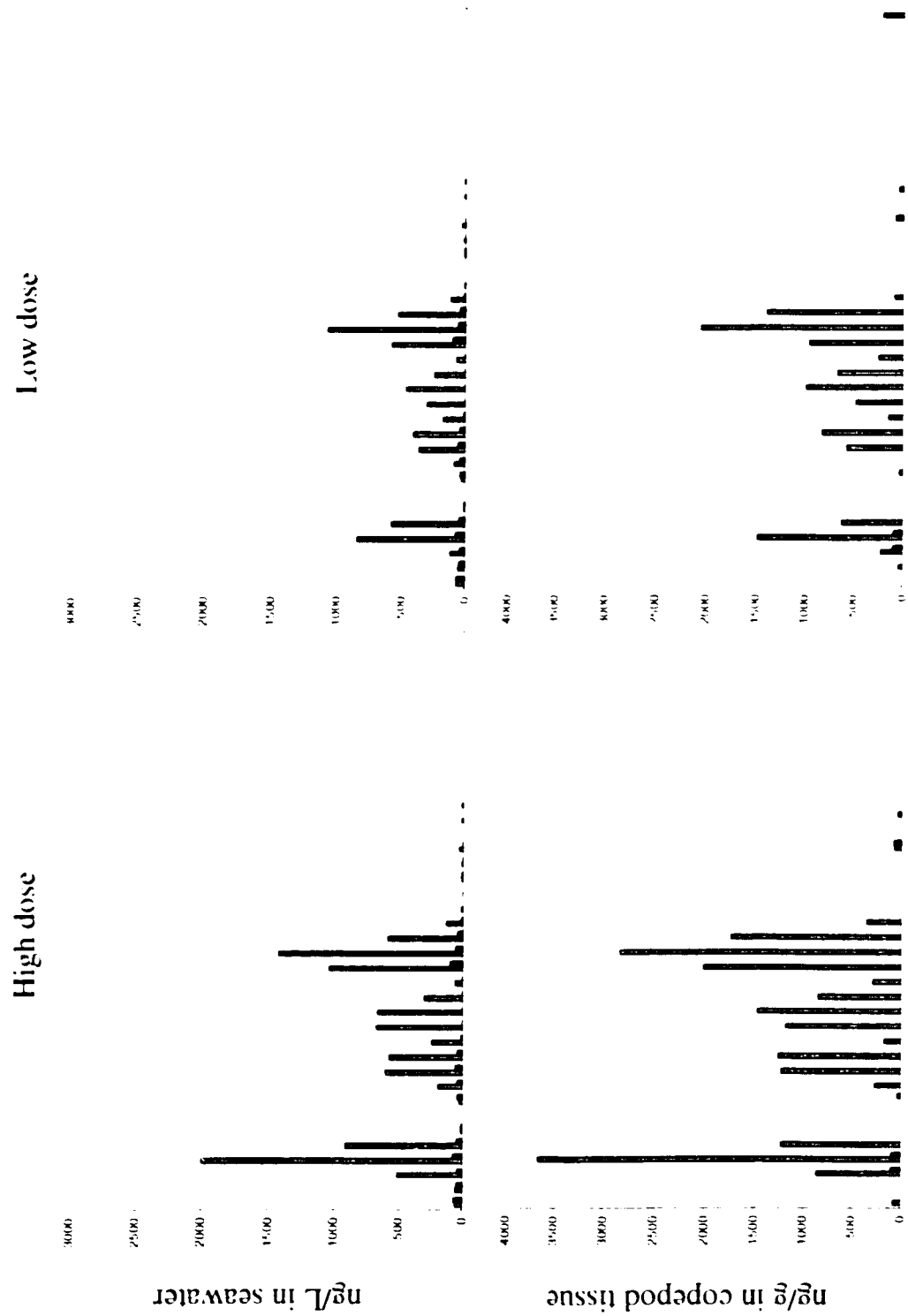


Figure 2.2: Proportions of the major lipid classes in *Neocalanus flemingeri* and *N. plumchrus*

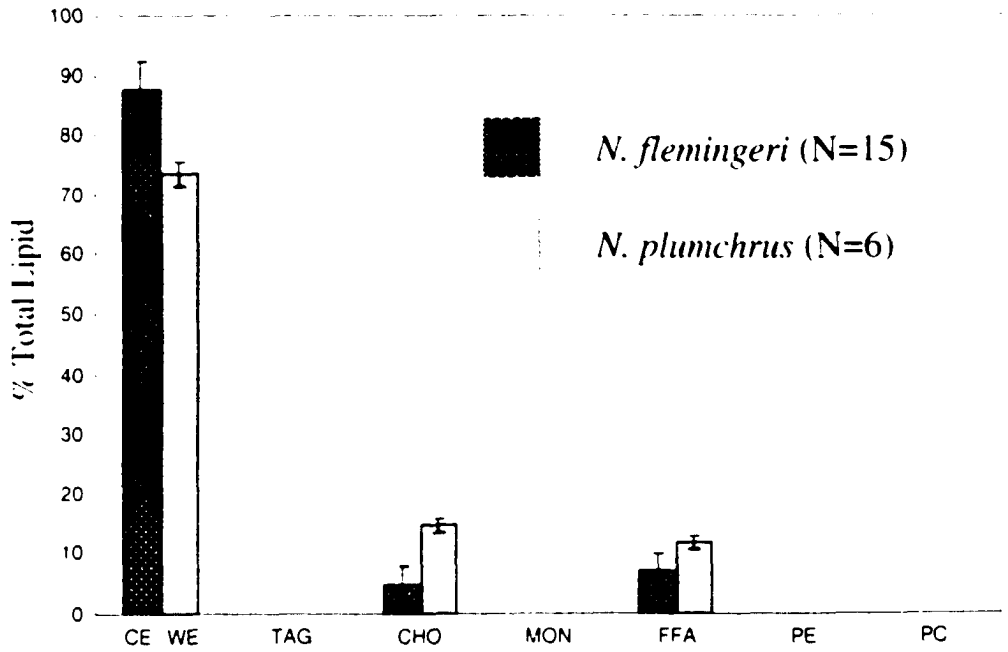
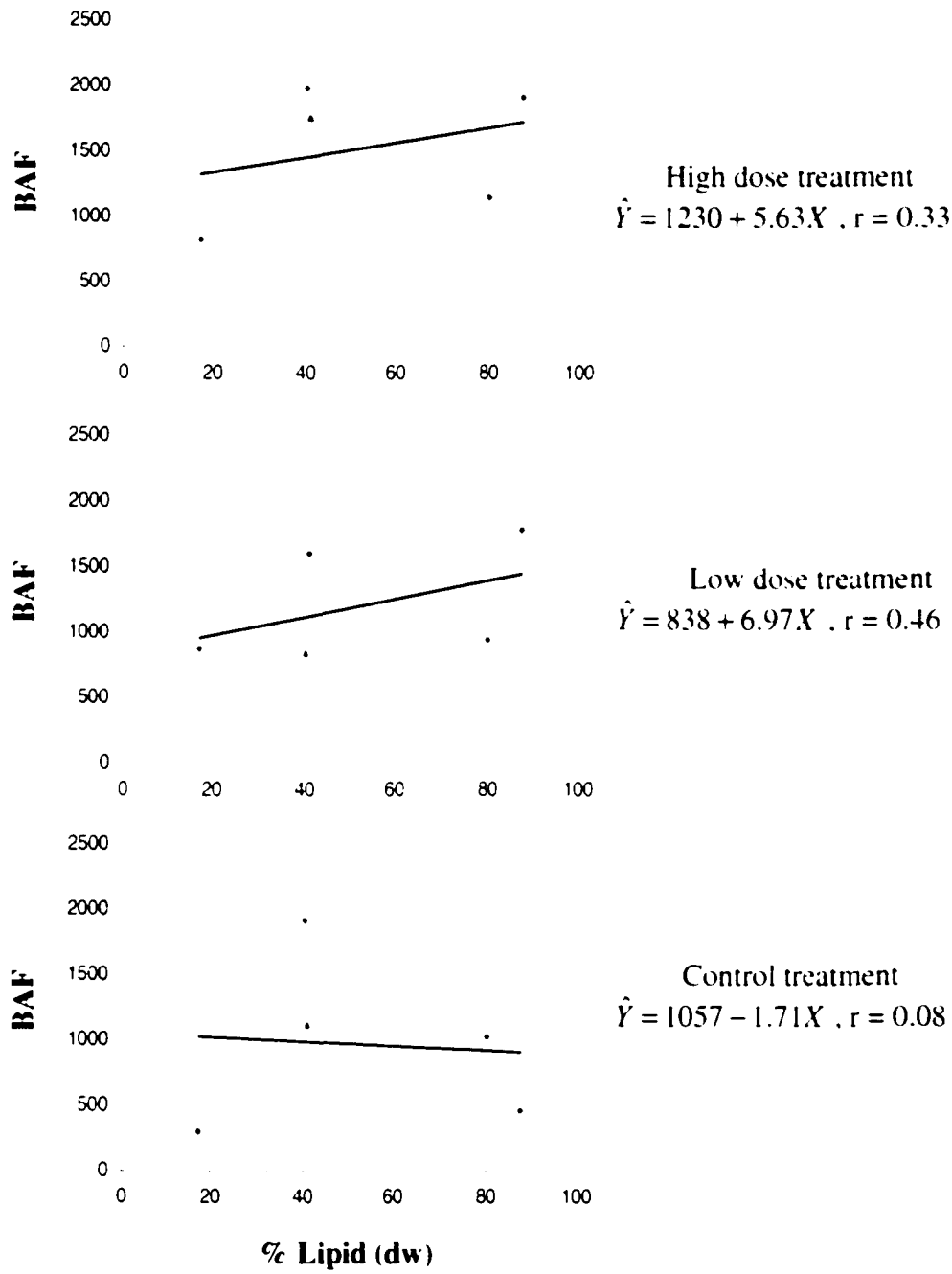


Figure 2.3: Correlation between bio-accumulation factors (BAF) and total lipid content in *Neocalanus* copepods in High dose, low dose and no oil treatments.



Chapter 3

PHOTOENHANCED TOXICITY OF WEATHERED ALASKA NORTH SLOPE CRUDE OIL TO THE CALANOID COPEPODS *CALANUS MARSHALLAE* AND *METRIDIA OKHOTENSIS*

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ABSTRACT

This study investigated the synergistic toxicity of aqueous polyaromatic compounds (PAC) dissolved from crude oil and ultraviolet radiation (UV) in natural sunlight to the calanoid copepods *Calanus marshallae* and *Metridia okhotensis*. These copepods were first exposed to low doses (~2 µg total PAC/L) of the water-soluble fraction of weathered Alaska North Slope crude oil for 24 hours and subsequently to low or high levels of natural sunlight. Responses included mortality, impairment of swimming ability and discoloration of lipid sacs. There was 80 to 100 % mortality and morbidity of *C. marshallae* exposed to sunlight and oil, compared to less than 10 % effect in oil only or UV only treatments. In *M. okhotensis* 100 % mortality occurred in the sunlight and oil treatment, 43 % mortality and 27 % morbidity in the sunlight only treatment, and less than 5 % effect in the oil only treatment. Bioaccumulation factors were ~8000 for *C. marshallae* and ~2000 for *M. okhotensis*. The interaction of the effect of PAC and UV radiation was highly significant ($P < 0.005$) in both experiments.

INTRODUCTION

Toxicological studies used to define the hazards of polycyclic aromatic compounds (PAC) and oil have usually been conducted under laboratory lighting with minimal ultraviolet radiation (UV) (*1*). Recent studies have established that PAC derived from petroleum sources cause toxicity to aquatic fauna that is enhanced 2 to greater than 100 fold by exposure to UV (*2*),(*3*). Photoenhanced toxicity occurs when organisms are exposed to the UV component of sunlight following tissue accumulation of PAC

(photosensitization) or when dissolved PAC are photochemically transformed to compounds of higher toxicity and subsequently absorbed by the organism (photomodification). Some PAC can catalyze the production of electronically excited molecular oxygen by transfer of energy initially absorbed by the PAC from UV (*4*),(*5*). The excited oxygen may then increase rates of non-specific oxidation within tissues. Translucent biota inhabiting the upper water column or the intertidal and shallow subtidal epibenthos are exposed to UV in sunlight, and may encounter PAC dissolved from chronic or catastrophic oil pollution sources.

Previous laboratory studies have used laboratory cultured test species, lengthy durations of UV exposure (e.g., 30 to 100 hours), and aqueous PAC extracts prepared by slowly stirring fresh- or seawater beneath thick surface slicks of oil where the ratio of surface area to volume of the oil $(S/V)_{oil}$ is relatively low (~ 2 cm^{-1}). These mixing conditions favor PAC extracts that are especially enriched in smaller PAC that dissolve more rapidly (e.g. naphthalene homologues (*6*)), but are not phototoxic. In contrast, the $(S/V)_{oil}$ of oil slicks resulting from oil spills are usually much higher (~ 200 cm^{-1}) than those typically used to prepare laboratory test solutions. This higher relative surface area accelerates the dissolution rates of PAC, which can lead to higher concentrations of three and four ring PAC, some of which are phototoxic. We therefore conducted experiments reported below to investigate the photoenhanced toxicity of PAC extracted from weathered crude oil under conditions of high $(S/V)_{oil}$ to two ecologically important and vulnerable marine species of calanoid copepods, under environmentally realistic exposure conditions.

Calanoid copepods occupy an important niche in marine food webs because they ingest a substantial proportion of annual primary production in the temperate and subarctic waters of the North Atlantic and North Pacific oceans (*7*), and so account for the majority of the secondary production on a biomass basis in these waters. Copepod abundance also influences the density and composition of phytoplankton through grazing and nutrient recycling (e.g.(*8*)). As secondary production they are prey for most of the higher-trophic level species, either directly or indirectly; e.g. forage or juvenile fishes are usually zooplanktivorous, and are themselves prey for piscivorous fishes and marine mammals. The most ecologically important genera of the calanoid copepods in this respect include *Calanus*, *Neocalanus*, *Metridia*, and *Pseudocalanus* and can constitute > 60 - 70 % of zooplankton biomass (e.g. (*8*)). These copepods are all translucent, and the advanced life stages of many are exposed to UV light while grazing on phytoplankton blooms near the seasurface during daylight (*9*). Many of these copepods, especially in the genera *Calanus* and *Neocalanus*, accumulate stores of lipids in their later life stages that may exceed 60 % of their dry weight (*10*),(*11*),(*12*), and may bioaccumulate substantial burdens of lipophilic pollutants such as PAC through equilibrium partitioning. Calanoid copepods thus include ecological key-role species that may be especially vulnerable to photoenhanced toxicity of PAC derived from petroleum products.

In this study we evaluated the photoenhanced toxicity of low doses (~2 µg total PAC/L) of weathered Alaska North Slope crude oil to two species of calanoid copepods, *Calanus marshallae* and *Metridia okhotensis* field collected in Alaska waters. A new continuous

flow PAC-exposure system was developed for these exposures. Exposure durations to PAC solutions and subsequently to natural sunlight at a subarctic latitude were less than 24 and 8 hours, respectively, and included one test where the natural sunlight was attenuated by cloud cover during a rainy day. These exposure conditions and test species all occurred concurrently during the *Exxon Valdez* oil spill in Prince William Sound, Alaska, so our experiments simulate conditions that may be encountered in the field.

METHODS

We performed two successive experiments, which were identical in all respects except for the UV exposure and the number of copepod species used. A 24 hr exposure to oiled seawater treatments in an indoor flow-through-system was followed by a static outdoor UV exposure. Each experiment included three treatments: (1) exposure to sunlight radiation but no exposure to PAC (denoted as "UV only")(Fig.1, treatment 1 a+b), (2) exposure to PAC dissolved from crude oil but no exposure to sunlight radiation (denoted as "Oil only")(Fig.1, treatment 2 a+b), and (3) exposure to PAC followed by exposure to sunlight radiation (denoted as "Oil+UV") (Fig.1, treatment 3 a+b). In each experiment one composite sample of copepods was frozen immediately following the PAC exposure for later evaluation of tissue PAC content (Fig. 1, treatment 4 a+b). Copepods in the oil treatments were exposed to about 2 µg total PAC/L in both experiments. In experiment 1 (high UV experiment) we exposed 15 to 16 *C. marshallae* per flask to bright natural sunlight for 3.8 hours. In experiment 2 (low UV experiment) we exposed 22 to 23 *C. marshallae* per flask and 31 and 40 *M. okhotensis* per flask to 8.2 hours of cloud-

attenuated sunlight. The number of copepods per flask differed because of variable availability of freshly caught organisms. After the UV exposure all copepods were evaluated for their biological response, then transferred to clean seawater and checked for delayed effects the following day.

Animal Collection

C. marshallae and *M. okhotensis* were collected in Lynn Canal and adjacent Barlow Cove in southeastern Alaska. They were pipetted from zooplankton collected with a 330 µm mesh plankton net towed vertically from a maximum depth of 120 m. The fifth copepodite stage (CV) of *C. marshallae* was identified by microscopic examination and selected for testing. Live specimens of *M. okhotensis* were not distinguished to life stage, but the population sampled contained copepodites at least 3.5 mm long (total length), and consisted mostly of stage CV and adults. Copepods were subsequently stored at 6 °C for about 24 hours until start of the oil exposure.

Oil Exposure

The Alaska North Slope crude oil was weathered by heating and overnight stirring at 80 °C to 20 % weight loss, which removed most monocyclic aromatic compounds, then added to 3 mm diameter glass beads at an application rate of 2.6 g oil/kg beads and tumbled for approximately 24 hours. The oiled beads were spread to single layer and left under a hood for 4 days at 25 °C to allow the oil to harden onto the beads, and then were stored at - 20 °C until use.

Three generating columns that produced aqueous solutions of PAC dissolved from crude oil were constructed by placing 100 mL of the oiled beads inside 25 cm long by 2.5 cm ID glass columns stoppered at each end by a glass plug and a piece of plankton mesh (505 µm) followed by a neoprene stopper penetrated by a 2.8 mm ID glass tube. For UV only treatments a separate column was constructed in the same way except that the glass beads were not coated with oil. A dilute solution of PAC was prepared by pumping natural seawater (30 ‰, 10 ± 1°C) through the columns at 5 ± 0.5 ml/min flow rate by peristaltic pump. The effluent from the columns was directed through glass tubing into 2 L Erlenmeyer filtration flasks for 20 h to rinse the exposure apparatus, followed by introduction of 15 - 40 copepods to each flask, depending on species and experiment. The ratio of total copepod wet weight and the exposure volume was less than 0.05 g/L. The exposure flasks were fitted with a small nylon screen to prevent copepod escape, and samples of flask effluents were collected at the start and end of each experiment to measure PAC doses. At the end of the oil exposure copepods collected for analysis of tissue PAC content (Fig. 1, treatment 4a+b) were immediately frozen at –20°C. Oil exposures and all handling procedures were conducted under fluorescent indoor lightning with negligible UV.

UV exposures

For the UV exposures, test flasks were placed in an outdoor waterbath (10 ± 1°C). In experiment 1 the temperature in the flasks was 10 ± 1°C, in experiment 2 the temperature was 14 ± 1°C. The difference corresponded to the difference in water temperature in

Auke Bay. Dissolved oxygen in the flasks was not measured in experiment 1, in experiment 2 it was 17 ± 0.01mg/L for all treatments of *C. marshallae* and 13.2 - 14.6 mg/L in the *M. okhotensis* flasks. The dissolved oxygen content of saturated seawater was 18.4 mg/L. Water levels in the flasks and waterbath were approximately equal. Attenuation of light by the borosilicate glass flasks was 3 % for visible light, 16 % for UVA and 64 % for UVB. The percentage attenuation from the flask was determined from the difference in UVA, UVB, and visible light measured by an Optics S2000 photodiode array spectrometer (Ocean Optics, Inc., Dunedin, FL) inside and outside the flask. The fiber optic cable with a cosine correcting diffuser was inserted through a small hole drilled in the bottom of the flask, so that the surface of the diffuser was slightly above the bottom of the flask. Measurements were made in rapid succession under natural sunlight during cloudless conditions to ensure constant solar radiation. The oil-only exposure flasks were wrapped in aluminum foil to exclude all light. High UV exposures (experiment 1) were conducted on July 13 (12:38 to 16:27), and low UV exposures (experiment 2) were conducted on July 19 (11:38 to 19:47), 2000 in Juneau, Alaska. July 13 was cloudless, and July 19 was heavily overcast with 1.4 cm rainfall. The duration of the low UV exposure was longer to provide approximately 50 % of the high UV exposure. UV intensity was continuously monitored at the water bath level with a five channel radiometer (GUV-511: Biospherical Instruments, San Diego, CA) linked to a computer. Average UVA (320 to 400 nm) and UVB (280 to 320 nm) intensities ($\mu W/cm^2$) during sunlight exposures were estimated by summing the measured UV intensity for each channel and the interpolated UV intensity outside of each bandpass.

Total UV dose ($\mu W*hr/cm^2$) was determined from the average UV intensity and the duration of sunlight exposure.

Biological response measurement

Immediately following UV exposure, all copepods were individually evaluated. If the initial escape response was slow, the copepods were investigated microscopically with regard to swimming behavior and mobility of appendages. Copepods were categorized as unaffected, impaired in their swimming ability, or dead. Impaired copepods were unable to move their antennules, or had no use of antennules and pleiopods. Affected individuals were examined microscopically for evidence of tissue damage. Subsequently, all live specimens were transferred to filtered seawater. The evaluation procedure was repeated 17.5 hours after the end of the high UV and 22.5 hours after the end of the low UV exposure.

Chemical analysis

Procedures for the quantitative determination of PAC in water and in tissues were described by Short et al. (*13*). Seawater samples (0.9 L) were extracted twice with 100 mL dichloromethane. Copepods were pulverized in a porcelain grinder three times each with 1 mL dichloromethane. Dichloromethane extracts of the PAC were reduced in volume and exchanged with hexane over a steam bath, followed by fractionation and purification by alumina/silica gel chromatography. PAC were measured by gas chromatography/mass spectrometry (GC/MS) in the selected ion monitoring mode (SIM).

PAC analytes included dibenzothiophenes and polyaromatic hydrocarbons containing 2-5 rings, including the alkylated homologues listed in Figure 2.

All tissue concentrations above method detection limits are reported on a dry-weight basis. A method blank, spiked method blank, and two reference samples were analyzed with each batch of 12 samples to verify method accuracy, precision, and absence of laboratory introduced artifacts and interferences. Detection limits were determined experimentally (*14*) for PAC and generally were 5 - 20 ng PAC/L seawater or 0.05 - 0.2 ng PAC/copepod. Concentrations below the detection limit were treated as 0.

Data analysis

Concentrations of total PAC were calculated by summing the concentrations of each of the PAC above the method detection limit. The relative concentrations of PAC were calculated as the ratio of each respective PAC concentration to the total PAC concentration. Water samples were collected at the beginning and end of the exposure period from each generating column in each experiment. Means of PAC in these samples are presented in Fig. 2(a), where samples from columns 2, 3 and 4 (Fig. 1) were treated as replicates (total of 12 samples). The differences in total PAC measured at the beginning and end of each experiment were evaluated by Student's t-test. Biological response data were tabulated as proportions and 95 % confidence limits for proportions were derived from binomial confidence limit tables (Fig. 3) (*15*). The use of the binomial distribution requires the assumptions that each copepod has an equal probability of death from the

treatment and that copepod deaths are independent of each other. Significance of the interaction of the effects of oil and UV exposure was tested with multivariate contingency tables using Chi-square statistics; the hypothesis of independence was tested at the 95 % significance level (*16*). For the purpose of this test, biological response data were grouped into "unaffected" and "affected" (impaired or dead).

The concentrations of PAC in copepods were calculated as the ratio of the amount of analyte per individual and the dry weight of individuals given in the literature. We used 175 µg/ind. for the dry weight of *C. marshallae* CV (from (*8*)). For *M. okhotensis* we assumed a mean stage of CV, and used a wet weight per individual of 1.48 mg (*17*) and a ratio of dry to wet weights of 18.8 % (*18*; table 4) to estimate a dry weight per individual of 278 µg/ind.

RESULTS

PAC Exposures and uptake

The solution of PAC in seawater produced by the oil exposure apparatus (Fig. 1) consisted mainly of three-ring PAC (Fig. 2(1)), at total PAC concentrations near 2 µg/L. The total PAC concentration increased from 1.55 ± 0.65 µg/L (n = 3) at the beginning of experiment 1 to 2.64 ± 0.38 µg/L (n = 3) at the end, a marginally significant (P = 0.051) change. During experiment 2 the total PAC concentration change was insignificant (P = 0.77) and averaged 2.08 ± 0.43 µg/L (n = 6). In the aqueous phase exposures, known

phototoxic PACs such as anthracene, flouranthene, and pyrene had low concentrations relative to the measured total PAC.

Both *C. marshallae* and *M. okhotensis* accumulated high concentrations of total PAC from the exposure seawater. The histogram in Fig. 2(b) shows the PAC proportions in tissues in one sample of *M. okhotensis* and two samples of *C. marshallae* taken at the end of the 24 hour oil exposures. The total PAC concentrations in *C. marshallae* were 85.4 µg/g dry weight and 71.7 µg/g dry weight at the end of the oil exposure of experiments 1 and 2, respectively, compared to a concentration of 21.3 µg/g dry weight in *M. okhotensis* at the end of the oil exposure in experiment 2. The composition of the PAC accumulated by the copepods was nearly identical with the PAC composition of the exposure seawater (Fig. 2). The apparent bioaccumulation factor was approximately 8000 on a wet weight basis for *C. marshallae* (averaged for the two samples) and approximately 2000 for *M. okhotensis*.

UV Exposures

Experiment 1 (high UV exposure) had four to five times the UVA and UVB intensity of experiment 2 (low UV exposure), and approximately 2 times the UV dose because of the shorter exposure duration (Table 1).

Photoenhanced toxicity to C. marshallae

In experiment 1 (high UV exposure) more than 80 % of the *C. marshallae* copepods exposed to Oil+UV were affected. Of these 60 % were impaired or immobile and 20 % were dead. In contrast, none of the copepods were affected when exposed to oil only or UV only (Fig. 3a). Two-thirds of those copepods impaired or immobile at the end of the UV exposure died by the following day in this experiment (6 animals), while one other impaired copepod recovered (Fig. 3b).

In experiment 2 (low UV exposure), no immediate mortality was observed for *C. marshallae* in the Oil+UV treatment, but about 85 % were immobile by the end of the UV exposure (Fig. 3c). Only one copepod immobile at the end of the UV exposure died by the following day, while three immobile copepods recovered the ability to move their swimming appendages but not their antennules (Fig. 3d). In contrast, less then 15 % of the copepods were affected when exposed to oil only or UV only, similar to the high-UV experiment (Fig. 3a,c). *C. marshallae* in the Oil+UV treatment that were impaired or immobile had opaque rather than transparent lipid sacs, whereas copepods in the oil only or UV only treatments had transparent lipid sacs (Fig. 4).

Photoenhanced toxicity to M. okhotensis

The pattern of results for *M. okhotensis* was similar to that of *C. marshallae*, except a higher UV sensitivity was observed (Fig. 3e). All copepods (n = 31) in the Oil+UV treatment were dead by the end of the UV exposure. In the UV only treatment (n = 40) 70

% were affected (17 died and 11 were either immobile or impaired in their swimming ability) with 12 copepods being unaffected. Five percent were affected in the Oil only treatment (n = 31).

For all experiments, interactions between oil and UV exposures were highly significant (Chi-squared test, $P < 0.005$), clearly indicating photoenhanced toxicity.

DISCUSSION

Phototoxicity of Alaska North Slope crude oil

Our results demonstrate that PAC dissolved from crude oil are phototoxic to subarctic marine copepods at aqueous PAC concentrations that would likely result from an oil spill, and at UV radiation intensities that would often be encountered in nature. Although our experimental treatments were not replicated within the high- or low-UV experiments, the results of the two sequential experiments with *C. marshallae* may be considered as a duplicated experiment in which one of the dosage treatments (UV exposure) is affected by a random variable (sunlight intensity). The highly significant interaction between oil exposure and subsequent UV exposure in both experiments is compelling evidence of photoenhanced toxicity to *C. marshallae*. The similar pattern of results with *M. okhotensis* is additional confirmation of these effects, and suggests that different species of translucent copepods may be vulnerable to photoenhanced toxicity if exposed to environmentally realistic concentrations of PAC and UV radiation in the upper water column. For example, total PAC concentrations exceeding 2 µg/L have been measured

beneath oil slicks following catastrophic oil spills (*19*),(*20*),(*21*) and the UV intensities in the present study were within the expected ranges for the environmental conditions, time of year, and latitude of the exposures: The average UVA intensity of the high UV exposure (3,700 $\mu W/cm^2$) is equivalent to 18 % attenuation of surface sunlight at solar noon on a clear day in Prince William Sound, Alaska (*22*). The low UV exposure (average UVA intensity of 761 $\mu W/cm^2$) is comparable to UVA levels reported for highly attenuated aquatic habitats (*23*). UVA appears to be the most active region of the light spectrum for photoenhanced toxicity, whereas UVB appears responsible for the majority of biological injury from UV only exposure (*24*).

Photoenhanced toxicity has been reported from single compound studies for several aquatic organisms (e.g. (*2*),(*4*),(*25*),(*26*),(*27*),(*28*),(*29*)) but limited literature is available on phototoxicity of crude or refined oils (*30*). While lethal concentrations of total PAC to some copepod species are reported on the order of 0.05 to 9.4 mg/L, we found phototoxic concentrations to be lower by a factor of 23 to > 4000 (*31*). Phenanthrenes were the most abundant PAC analyzed in copepod tissue in this study and, like flourenes and naphthalenes, are not photosensitizers (*1*),(*32*),(*33*). Photoproducts of phenanthrenes, however, were more toxic than the parent compound in tests with the diatom *Phaeodactylum tricornutum* (*34*). Flouranthenes and pyrenes are highly phototoxic to bivalves and mysids (*2*) but their concentrations were below the method detection limit in copepods (Fig. 2). The phototoxicity of dibenzothiophenes is uncertain, and chrysenes are

the only known phototoxic agents detected in copepod tissue in this study. Clearly more work is needed to identify the phototoxic compounds in crude oil.

Bioaccumulation of PAC

Organisms with a high ratio of surface area to volume rapidly accumulate PAC (*35*). The similarity of the PAC compositions in the exposure water and in the copepod tissue at the end of the exposure indicates that the PAC were passively accumulated from the water without selective uptake (Fig. 2). Copepods may thus form an important and largely unrecognized ecological compartment for the accumulation of PAC from the water and the transfer of PAC to higher trophic level consumers. The higher PAC concentrations found in *C. marshallae* (accumulation factor ≈ 8000) compared with *M. okhotensis* (accumulation factor ≈ 2000) may reflect different sizes of lipid pools. Bioaccumulation of polyaromatic hydrocarbons (PAH) in *Daphnia pulex* was described in terms of lipid/water PAH partitioning and a linear relationship between the n-octanol- –water partition coefficient and the concentration factor for several PAH was reported (*35*). Consistent with the results for the marine copepods, accumulation factors of PAHs in *D. pulex* ranged from 100 to 10,000 and equilibrium concentrations were approached within 24hr exposure periods (*35*). Both copepod species have high surface area to volume ratios and would therefore approach equilibrium rapidly. But *Calanus* and *Neocalanus* copepods have higher bioaccumulation capacities for PAC because of their characteristically higher total lipid content. Most estimates for lipid content in *Daphnia*

fall in the 1-3.5 % range(*35*), while total lipid content in high latitude copepod species varies seasonally from 8 % to 73 % (*8*).

Tissue damage

In many copepods lipid is accumulated during the spring feeding season and stored internally as wax esters (*36*). The highly lipophilic PAC are accumulated by organisms and absorbed into fat tissue (*4*). The opaque appearance of the lipid sac of *Calanus marshallae*, observed in the Oil+UV treatment may be indicative of severe lipid damage resulting from lipid peroxidation caused by singlet oxygen as has been shown in fish (*5*). In comparison, copepods in the Oil only and UV only treatments had transparent lipid sacs (Fig. 4). Light scattering occurs when molecular clusters in the path of the light are larger than the wavelength of the penetrating light. Thus, formation of larger molecular structures must have occurred in the lipid sac or its surrounding membrane to cause the observed change from a transparent to cloudy appearance. This observation may hold promise for a method to assess damage from photoenhanced oil toxicity to surface feeding copepod swarms in their natural environment. More research is necessary to determine whether the tissue damage from lipid peroxidation is consistent and discernible in preserved samples. Also, although this interpretation suggests a photosensitization mechanism, our experiments were not designed to explain the mechanism of phototoxicity involved. Photosensitization was identified as the main mechanism causing photoenhanced toxicity in various invertebrates (*2*),(*28*),(*32*),(*37*), but several authors described photomodification to cause increased toxicity of PAC to plants (*34*),(*38*),(*39*).

Ecological implications

Phototoxic effects on copepods could conceivably cause ecosystem disruptions that have not been accounted for in traditional oil spill damage assessments. Particularly in nearshore habitats where vertical migration of copepods is inhibited due to shallow depths and geographical enclosure, phototoxicity could cause mass mortality in the local plankton population. This lack of primary consumers could trigger initial increases in phytoplankton, but food depletion in juvenile or forage fish populations (e.g. salmon fry). The observed sublethal response of copepods to phototoxicity was an impairment of the escape response which may cause increased vulnerability to predators. The potential for photoenhanced toxicity to fish larvae from transfer of PAC from prey is currently unknown, although feeding on oil-contaminated prey in pink salmon fry has been reported to reduce growth rates (*40*). Areas of future research should include identifying phototoxic compounds and quantifying injury to copepod populations.

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Figure captions

Figure 3.1: Diagram of the flow-through oil exposure system. Seawater is pumped from an overhead tank through generators filled with oil coated glass beads to achieve consistent low dosage exposure of organisms in Erlenmeyer flasks. Generating columns 2,3 and 4 contain oiled glass beads, column 1 contains unoiled beads for control treatment. In experiment 2 the effluent from Erlenmeyer flasks 1a, 2a, 3a and 4a is directed into Erlenmeyer flasks 1b, 2b, 3b and 4b, respectively.

Figure 3.2: (a) Proportions of PAC analytes in exposure seawater (N = 6 replicate samples; these were collected at the start (N = 3) and end (N = 3) of the 24 hr exposure time from generators 2, 3 and 4; see Fig. 1), ranges are depicted by bars but are very small (between 0 and 4%).

(b) Proportions of PAC analytes in 2 samples of *C. marshallae* (white bars = high UV, black bars = low UV) and 1 sample of *M. okhotensis* (gray bars), samples were collected before UV exposure; n = sampling units in samples; N, F, D, P and C refer to naphthalene, flourene, dibenzothiophene, phenanthrene and chrysene, respectively, and the numbers following these letters indicate the number of alkyl-substituent carbon atoms. Other PAC are abbreviated as follows: bip = biphenyl, ace = acenaphthylene, acn = acenapthene, ant = anthracene, fla = flouranthene, pyr = pyrene, C1fp = C1 flouranthenes/pyrenes, baa = benz-a-anthracene, bbf = benzo-b-flouranthene, bkf =

benzo-k-flouranthene, bep = benzo-e-pyrene, bap = benzo-a-pyrene, per = perylene, icp = indeno-1,2,3-c,d-pyrene, daa = dibenzo-a,h-anthracene, bgp = benzo-g,h,i-perylene.

Figure 3.3: Biological responses in phototoxicity experiments: Dark gray bars: dead; crosshatched bars: Impaired = unable to move swimming appendages; bars depict binomial confidence limits. n=sampling units in sample; **a)** *Calanus marshallae*; 24 hr oil exposure followed by 3.8 hr UV exposure on a sunny day **b)** as in (a) but after 17.5 hr depuration **c)** *C. marshallae*; 24 hr oil exposure followed by 8.2 hr UV exposure on a rainy day **d)** as in (c) but after 22.5 hr depuration **e)** *Metridia okhotensis*; 24 hr oil exposure followed by 8.2 hr UV exposure on a rainy day.

Figure 3.4. Photos of *Calanus marshallae* oil sacs; **a)** after 24 hr exposure to oil only, **b)** after 8.2 hr exposure to low UV only, **c)** after 24 hr exposure to oil followed by 8.2 hr low UV exposure. Lipid sacs appear clear in a and b, opaque in c (arrows point to coloration of lipid sac).

Table 3.1: Summary of UVA and UVB intensity and total dose (duration * intensity) in the high and low sunlight exposures (measured in air).

Experiment	Environmental conditions	Exposure duration (hr)	Average intensity ($\mu W/cm^2$)		UV Dose ($\mu W*hr/cm^2$)	
			UVA	UVB	UVA	UVB
High UV	Sun, no clouds	3.8	3731	60.9	14,253	233
Low UV	Clouds, haze, rain	8.2	761	16.8	6202	137

Figure 3.1: Diagram of the flow-through oil exposure system.

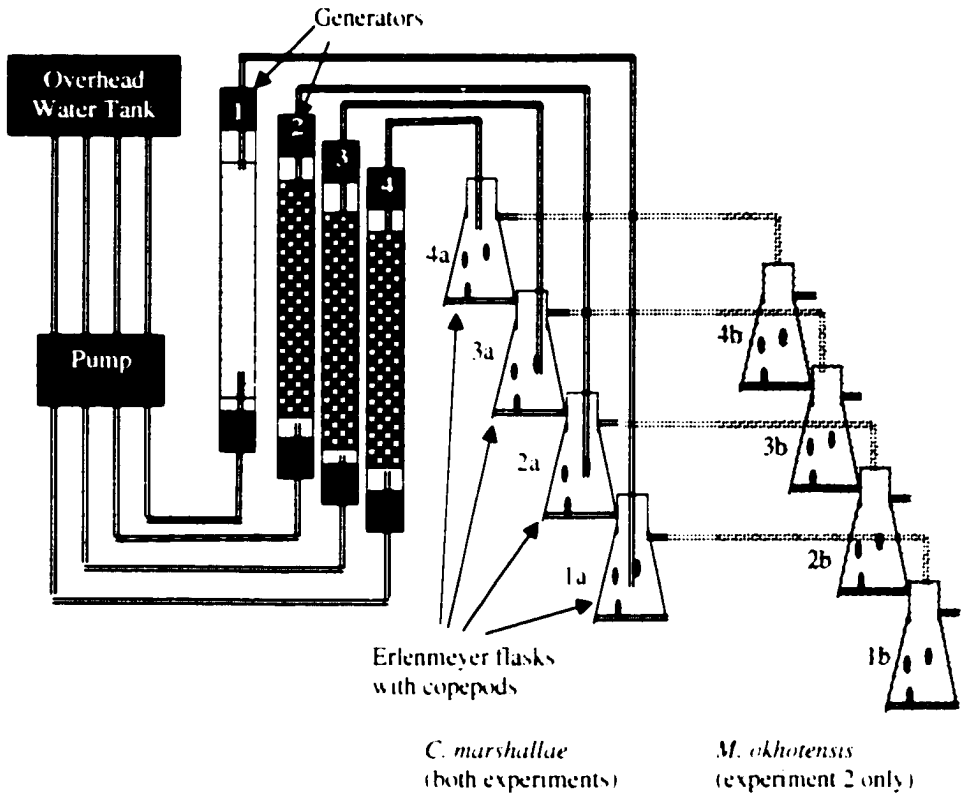

Figure 3.2: Proportions of PAC analytes in exposure water and copepod tissue.

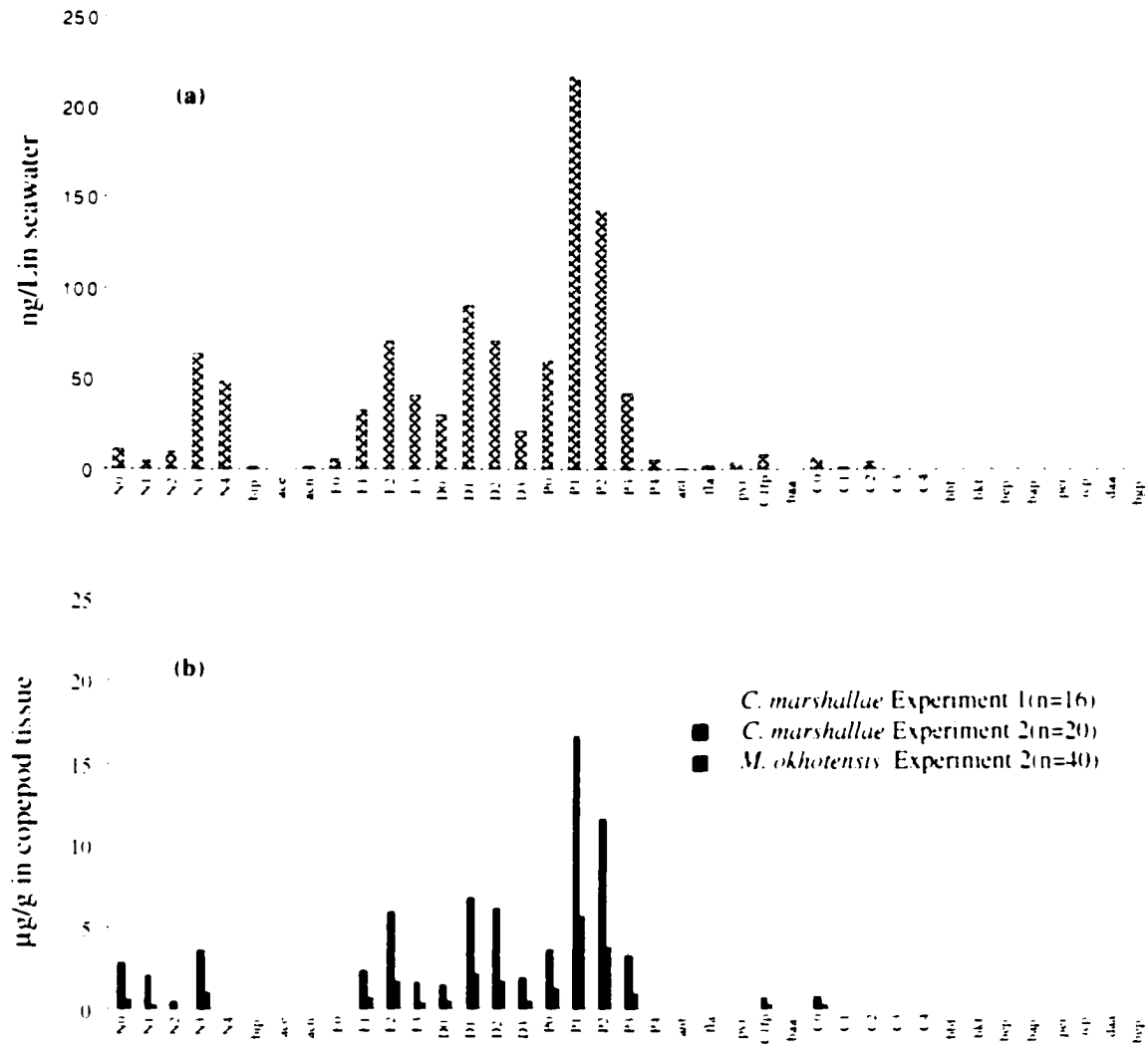

Figure 3.3: Biological responses in phototoxicity experiments.

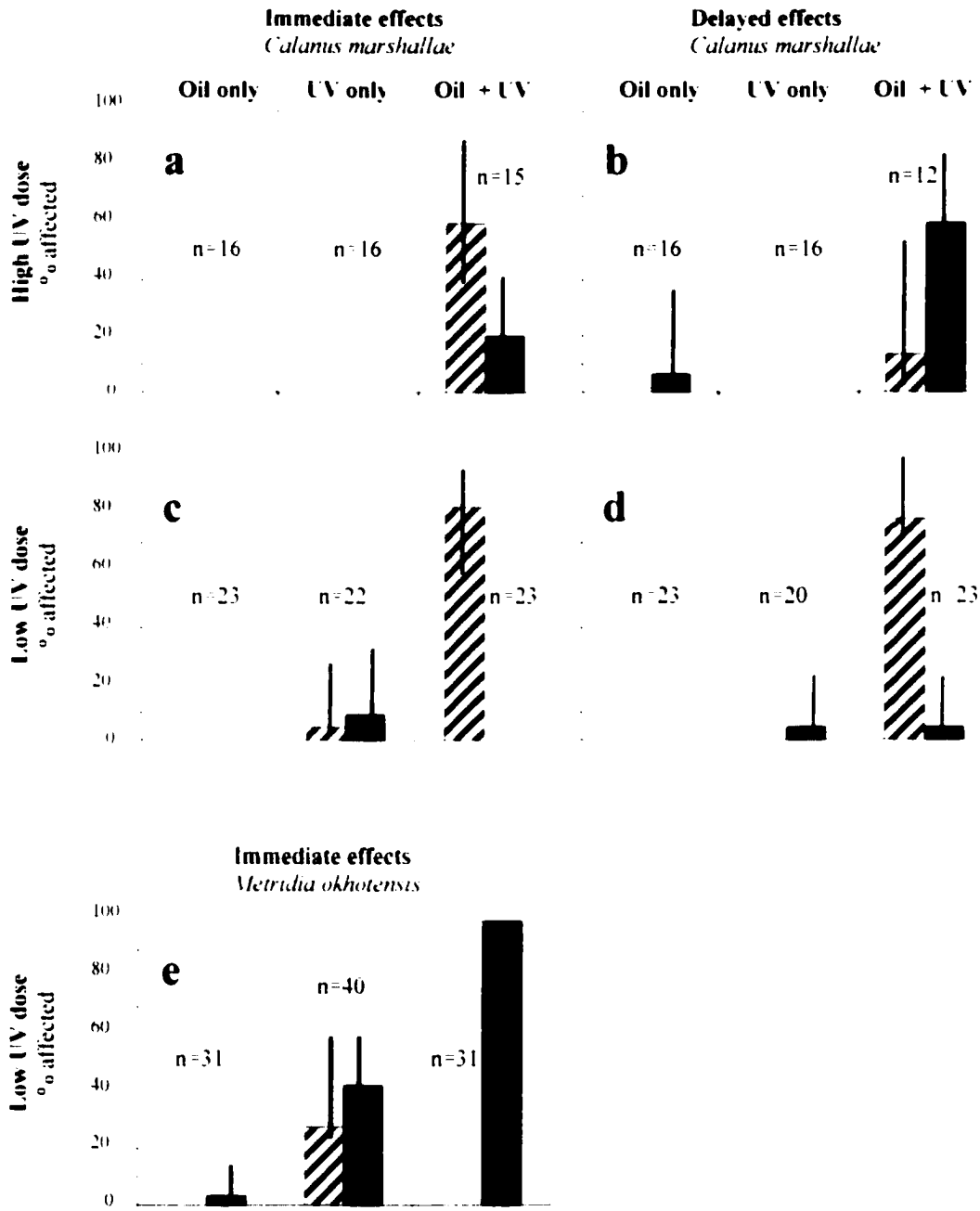

Figure 3.4: Photos of *Calanus marshallae* oil sacs.

Chapter 4

THE INTERACTION OF POLYAROMATIC COMPOUND (PAC) CONCENTRATION AND ULTRAVIOLET RADIATION DOSE IN PHOTOTOXIC EFFECTS ON *NEOCALANUS* COPEPODS IN THE NORTH PACIFIC

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ABSTRACT

Phototoxicity of dissolved Alaska North Slope crude oil to *Neocalanus* copepods was tested in experiments with concentrations ranging from 0.5 - 10 µg/L of total polyaromatic compounds (PAC) and UV radiation from ambient daylight exposures. The copepods were clearly sensitive to photoenhanced toxicity at these concentrations. The interaction of PAC and sunlight was highly significant ($P < 0.001$). No significant difference in effects existed between the full spectrum and the UVB-exclusion light treatments, indicating that UVA was the phototoxic region of wavelengths in these experiments. Phototoxic effects, recorded as the frequency of test copepods dead or impaired at the end of the UV exposure, were closely correlated with PAC concentration in the exposure water and the product of PAC concentration and light dose (intensity * exposure duration) in a linear relationship. This correlation was only slightly better when tissue residue concentrations were used ($r = 0.998$) than for water PAC concentrations ($r = 0.991$).

INTRODUCTION

Photoenhanced toxicity of oil in the presence of ultraviolet (UV) radiation has been shown for many single compound polyaromatic hydrocarbons and some crude oils and may occur at concentrations encountered in the environment (e.g., 1-3). However, the extent to which the effect occurs in waters contaminated with polyaromatic compounds (PAC) and the ecological consequences are not well understood. Most studies of phototoxicity have been conducted under artificial laboratory UV light and with oil

solutions with a relatively high proportion of lighter PAC compared to those created by weathered oil in the environment (4). In this study we tested the interaction effects of various concentrations of dissolved weathered Alaska North Slope crude oil, high in phototoxic PAC, and subsequent exposure to sunlight with and without the UVB component to the copepods *Neocalanus flemingeri* and *N. plumchrus*.

Copepods of the genus *Neocalanus* dominate the zooplankton biomass in the North Pacific, Gulf of Alaska and adjacent inlets during several months of the year (5). Their natural range includes clear ocean waters with maximum UV penetration properties, to highly productive inlets with ongoing oil exploration and high risk of PAC encounter. Many secondary consumers depend on *Neocalanus* as a large seasonal energy source, which is reflected in life history and behavior patterns that are geared towards the maximum chance of encountering the production peaks of these copepods. At 4 - 5 mm in prosoma length, late stage copepodites and adults of *Neocalanus flemingeri* and *N. plumchrus* are among the largest copepods and they can accumulate > 60 % of their body dry weight in lipid stores (6). Because of the lipophilic nature of oil-derived PAC, they accumulate in the lipid tissue of organisms and the high surface area to volume ratio of copepods accounts for a high bioaccumulation potential (4).

The toxicity of photoactivated PAC should be a direct function of chemical (PAC) dose and light intensity (7). Ankley et al. (1995) investigated the specific toxicity of fluoranthene to the benthic oligochaet *Lumbriculus variegatus* at three different light

intensities; a linear relationship between the product of light intensity and initial tissue PAC residue, and the median time-to-death was reported (8). The results supported the Bunsen - Roscoe photochemical law of reciprocity, which states that in the absence of "complicating " side reactions, the product of light intensity and reaction time is constant for a fixed concentration of the sensitizer (PAC). In a similar approach, we tested four PAC doses, while the light intensity and exposure duration were the same within each of the two experiments. Instead of reporting the time to death, we chose to record effects at approximately the time when half of the copepods in the medium PAC dose were affected. In this approach, the proposition was tested that the product of exposure duration and light intensity times PAC residue would follow a linear relationship when plotted against the percentage of affected organisms.

Known phototoxic PAC absorb in the UVA wavelength range (320-400 nm) (9). However, phototoxicity of weathered crude oil to larvae of the Pacific herring was increased when the exposures also included a UVB component (10). The role of UVB in phototoxicity to copepods was examined by including a light treatment with selective UVB exclusion to compare the phototoxic effectiveness of full spectrum sunlight and that of sunlight without the UVB component.

MATERIAL AND METHODS

Two separate experiments, each with three light spectra (no light, daylight without UVB, full spectrum daylight) and four concentrations of water dissolved PAC were conducted.

Oil exposures were conducted in the laboratory in 24 hour flow-through exposures and light exposures followed immediately after transfer to an outdoor waterbath. The oil exposure for experiment 1 was started on May 14, 2001, followed by daylight exposures from 15:20 to 19:10 on May 15 and 10:10 to 13:30 on May 16. For experiment 2, the oil exposure started on June 04, 2001, followed by daylight exposure from 11:45 to 16:25 on June 05. In experiment 1, 20 *Neocalanus flemingeri* were used per treatment. In experiment 2, 16 - 18 copepods were used per treatment, half of which were *N. flemingeri* and the others *N. plumchrus*.

Copepod collection

Copepods were collected with 200 µm mesh open ring nets, equipped with altered design cod ends to minimize breakage of setae, and towed vertically from 50 m depth to the surface. The live samples were kindly provided by researchers of the GLOBEC Gulf of Alaska Monitoring Program cruises, diluted if dense, and kept at ambient water surface temperatures until processed. In the laboratory, storage, sorting and experiments were conducted in a constant-temperature walk-in chamber set at 6 - 8 °C. Copepods were pipetted into 1 ml culture wells for microscopic species and life stage identification and quickly transferred to beakers with 5 copepods per 50 ml beaker until the start of the experiment.

Oil exposure and PAC analysis

The Alaska North Slope crude oil was weathered by heating and overnight stirring at 80 °C to 20 % weight loss, which removed most monocyclic aromatic compounds, then added to 2 mm diameter glass beads at application rates of 2.6 g oil/kg beads and tumbled for approximately 24 hours. The oiled beads were spread to single layer and left under a hood for 4 days at 25 °C to allow the oil to harden onto the beads, and then were stored at - 20 °C until use.

A detailed description of the generating columns that produced the aqueous solutions of PAC dissolved from crude oil is provided in Duesterloh et al. (4). The columns were filled with 20 -100 ml oil coated glass beads and unoiled beads were added to fill up excess volume. For the high dose treatments several generating columns were connected. In the control treatments the generating columns were filled with 100 ml PAC-cleaned glass beads. Prior experience showed that there was no loss in total PAC concentration from the columns within 96 hours. Seawater was directed from the laboratory supply line into an overhead tank of approximately 80 liter capacity. Water was then pumped through the generating column at a flow rate of 5 ml/min into a 2 liter Erlenmeyer filtration flask in which the hose connector served as an overflow. Each column was flushed with seawater for 22 hours before the peristaltic pump was activated and the flow rate in all columns was adjusted. The experiment was started within 20 hours after activation of the pump at which time 0.9 liter of the water in the Erlenmeyer flask was collected for PAC extraction and copepods were added to the remaining water volume in the flask. A screen

of 330 µm plankton mesh covered the outflow opening of the flasks to prevent loss of copepods. After 24 hours, copepods were collected and frozen (-20 °C) and 0.9 liter of the exposure water were extracted with dichloromethane and then frozen for later PAC analysis at the Auke Bay Laboratory in Juneau, Alaska (NMFS/NOAA).

Procedures for the quantitative determination of PAC in water and in tissues were described by Short et al. (11). Seawater samples (0.9 liter) were extracted twice with 50 - 60 ml dichloromethane. Tissue samples were collected in experiment 1 but not in experiment 2, because of lower *Neocalanus* abundance in the later sample. Copepods were macerated in a glass grinder twice, each time with 1 ml dichloromethane. Dichloromethane extracts of the PAC were reduced in volume and exchanged with hexane over a steam bath, followed by fractionation and purification by alumina/silica gel chromatography. PAC were measured by gas chromatography/mass spectrometry (GC/MS) in the selected ion monitoring mode (SIM). PAC analytes included dibenzothiophenes and polyaromatic hydrocarbons containing 2 - 5 rings. A method blank, spiked method blank, and two reference samples were analyzed with each batch of 12 samples to verify method accuracy, precision, and absence of laboratory introduced artifacts and interferences. Detection limits were determined experimentally (12) for PAC and generally were 5 - 20 ng PAC/L seawater at the 95 % confidence level. For tissues an 80 % confidence level was chosen. Concentrations below the detection limit were treated as 0.

UV exposures

For the UV exposures, a waterbath was installed outside and supplied with seawater from the laboratory intake lines. Temperatures were 7 ±1 °C (mean ± 1 standard deviation) during experiment 1 and 8 ±1 °C during experiment 2. Erlenmeyer flasks were placed in the waterbath so that the water level inside the flask was approximately the same as in the water bath, and arranged to avoid shading. For the flasks used for the UVB exclusion treatment, a Mylar-D cylinder was constructed to cover the entire flask. This reduced the UVB radiation by 68 % when measured in air compared to a reduction of UVA and visible light by 25 %. When measured in water and under the flask (measurements inside the flask were not possible because of the small neck), UVB was reduced by > 99 %, compared to a reduction of UVA and visible light of 15 - 17 %. All measurements were recorded with a Macam Photometrics UV meter at 10 cm depth in the water bath. The attenuation of 10 cm water was measured at 32, 33, and 21 % for UVB, UVA and visible light, respectively. Measurements of UVA, UVB and visible light were recorded every 10 minutes, then integrated to estimate total UV dose over the exposure period. The exposure was terminated when ~ 50 % of immobilization or mortality were observed in the medium dose treatment. Copepods were then microscopically evaluated and biological responses were categorized into unaffected, impaired and dead. For all further analysis the categories "impaired" and "dead" were pooled into one category "affected".

Statistical analysis

To test for a difference between start and end PAC concentrations in the exposure water,

a paired comparisons t-test was used. For experiment 2, a t-test (alpha = 0.05) was employed to test for a difference in effects on *Neocalanus flemingeri* and *N. plumchrus*.

Frequencies of affected and unaffected copepods were tabulated in three-dimensional contingency tables with the factors "PAC concentration", "light spectrum" and "affected" or "unaffected" (13). The two experiments were analyzed independently of each other. First, a test for mutual interdependence was conducted. When the null hypothesis of no interaction between the factors was rejected, a test for partial interdependence between PAC concentration and light spectrum was performed. For all tests the Chi-square statistic at 95 % significance level was used.

To test the proposition of a linear relationship between the product of light dose (intensity * exposure duration) and PAC concentration, the product of light dose and PAC concentration in copepod tissue was plotted against the percentage of affected copepods and the correlation coefficients were calculated for both light spectra in experiment 1. Then, with pooled data of the two experiments, the PAC concentrations in water were plotted against the percentage of affected copepods and correlation coefficients were calculated. Finally, total light dose was multiplied by the total PAC content in water and plotted against the percentage of copepods affected in the corresponding treatment; correlation coefficients were compared to those obtained from water PAC concentrations alone.

A Student's t-test was used to test for a difference in effects of the UVB-exclusion and full spectrum treatments in both experiments (alpha = 0.05)

RESULTS

An increase of impairment and mortality with PAC concentration was observed in treatments with a combination of PAC and sunlight exposure in both experiments, clearly indicating dose-dependent photoenhanced toxicity of oil on *Neocalanus* copepods (Fig. 1). No difference could be detected between the phototoxic effects on the two species *N. flemingeri* and *N. plumchrus* in experiment 2 (t-test, alpha = 0.05, $P < 0.001$).

UV Doses and PAC Concentrations

The total light doses and ranges of light intensity are given in Table 1. In experiment 1, the total dose was ~ 30 % higher in UVA and visible light and ~ 15 % higher in UVB compared to experiment 2. Mean PAC concentrations in the exposure water were between 0.6 and 9 µg/L. The difference between start and end concentrations (Fig. 2) was insignificant (paired comparisons t-test, alpha = 0.05). Exposure concentrations were approximately 1000 times less than reported LD_{50} concentrations of oil to copepods (14). In all further calculations the mean concentration was used (Table 2). The first two concentrations listed in Table 2 were the "no oil" control treatments. The low but prevalent total PAC concentrations measured in the "no oil" controls do not reflect experimental cross-contamination but rather a background signature of creosote that was

in the intake water. Concentrations of any individual PAC from this background signature were ≤ 0.01 times those of the measured phototoxic agents.

Test for interaction between light spectrum and PAC concentration

The interaction between PAC concentration and light spectrum was highly significant, indicating that the toxicity of dissolved weathered Alaska North Slope crude oil is enhanced by photo-activation. The null-hypothesis of no interaction between any of the variables was rejected ($P < 0.001$). Also, the partial independence test between light spectrum and PAC concentration resulted in rejection of the null-hypothesis of no interaction ($P < 0.001$).

*Test for linearity of PAC concentration *light dose versus effect*

The product of PAC concentration in copepod tissue and the light dose (intensity * exposure duration) was a good predictor of effects on copepods (Figure 3). The correlation coefficient r was > 0.99 in both light treatments (Figure 3 e, f). This was not true for the product of PAC in water concentration times light dose; the product of PAC concentration in water and the light dose was slightly less correlated with the percentage of affected copepods ($r = 0.93$) than the PAC concentration in water alone ($r = 0.97$) in the full spectrum treatment (Figure 3 a, c). In the UVB exclusion treatment however, the product of PAC in water concentration and light dose had a slightly higher correlation coefficient ($r = 0.97$) than PAC in water alone ($r = 0.95$) (Figure 3 b, d).

Phototoxicity of full spectrum light versus UVB - exclusion

The full spectrum treatment consistently had slightly higher frequencies of affected copepods than the UVB exclusion treatment (Figures 1, 2). However, this trend was not significant in t - tests comparing the means of the two treatments (alpha = 0.05). No effect was observed in the "no oil" treatments, which indicates that all light exposures were below toxic levels of UV radiation alone.

DISCUSSION

We observed a dose-dependent, significant interaction of PAC dissolved from weathered Alaska North Slope crude oil and UV radiation in sunlight to *Neocalanus* copepods. In one experiment conducted on June 4 - 5, there was no difference (t-test, alpha = 0.05) in effects on the species *Neocalanus flemingeri* and *N. plumchrus*. The significant increases in frequencies of affected copepods with increasing PAC concentrations are in accord with results obtained in studies with other test organisms and under artificial UV light sources (e.g., 2, 15). The observed dose-dependent interaction of dissolved PAC at these low concentrations (0.5 - 10 µg/L) and the natural sunlight source confirm and extend earlier results with the copepods *Calanus marshallae* and *Metridia okhotensis* (4).

A reciprocity relationship between PAC concentration measured in tissue residue and light dose was supported by our data ($r > 0.99$). In two independent experiments, each with four PAC exposure concentrations, the response was tightly correlated with the product of light dose and PAC concentration in the exposure water, and also with PAC

water concentration. PAC accumulation by copepods can be assumed to be rapid and proportional to the PAC water concentration at the levels tested, but tissue concentrations had slightly closer correlations with phototoxicity effects than water PAC concentrations (Figure 3). Confirmation of the reciprocity relationship and application of the Bunsen - Roscoe photochemical law was provided by Ankley (8). Because of the difficulty in accurately defining the time of death or unrecoverable damage in copepods, we chose to terminate the experiment when approximately 50 % effects were observed in the medium dose treatment. In this approach the factor "exposure time" becomes fixed and the relationship to be tested is that of PAC concentration and light dose (intensity * exposure duration). This implies a possible overestimation of the lethal dose (100 % affected) in the high PAC concentration treatments, because the experiment was continued past the test organism's time of death. However, the lowest PAC concentration in experiment 1 had no significant phototoxic effect. Thus, we can define a lower and upper phototoxicity threshold in terms of the product of light dose [$W * m^{-2} * min^{-1}$] and the PAC concentration in water [µg/L] between ~ 100 - 600.

The results of the UVB - exclusion experiment were not significantly different from those of the full spectrum light treatment, indicating that the observed phototoxicity was induced in the UVA wavelength range. However, there was a tendency for the frequencies of affected copepods to be slightly lower in the UVB - exclusion treatment compared to the full spectrum treatment. This may be explained by the ~30 % UVA attenuation of the Mylar - foil. An alternative explanation is that the interplay of UVA

and UVB wavelengths increases the total phototoxic effects. Photo-active agents differ in peak absorbance spectra (9), and phototoxicity of some increases in the presence of shorter (UVB) wavelengths (16). A significantly higher phototoxicity of sunlight compared to a UVA treatment was reported for herring larvae in experiments using ~ 10 times higher concentrations of dissolved weathered Alaska North Slope crude oil (10). More research is needed to resolve the role of UVB radiation in phototoxic effects of oil on copepods, particularly at higher PAC concentrations.

The UV exposures of both experiments were conducted on days with intermittent disk-visibility. For comparison, the visible light curves for experiment 2 and a clear sky day, measured at the same location and under the same conditions are shown in Fig. 4. The light doses used in our experiments were well below the total irradiation on a sunny day. In experiment 1, the light exposure was on two subsequent days, interrupted by a 13 hour dark period. Repair mechanisms could reduce damage caused by UVB radiation in some copepods species during periods of visible light exposure (17). However, no such repair occurred during 24 hours of incubation in the dark (17). Thus, we assume that the effects observed at the end of the exposure on day 2 reflect the accrued phototoxicity of the total light dose received on two subsequent days.

Oil exploration and transportation in Alaska has been accompanied by much concern about environmental risks. Numerous studies, mostly generated by the *Exxon Valdez* oil spill in 1989, investigated ecological implications of catastrophic oil spills and chronic

contamination in these highly productive marine ecosystems (18, 19). The potential for phototoxicity as a complicating and accelerating factor was only recently recognized (20). The present study clearly demonstrated the potential for dominant, subarctic copepods to accumulate dissolved PAC from the water and their sensitivity to photoenhanced toxicity. Because copepods are important forage species and many fishes and invertebrates directly or indirectly depend on their abundance as a vital energy source, population shifts in copepods may change ecological patterns on a large scale. However, it is presently unknown to what extend phototoxicity could threaten copepod populations that have encountered dissolved PAC from chronic or accidental contamination.

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Figure captions

Fig. 4.1: Phototoxic effects (impaired or dead) of dissolved oil on copepods in experiment 1 (*Neocalanus flemingeri*; N = 20, top panel) and total PAC concentrations in the exposure water at the start and end of the oil exposure (bottom panel). Bars indicate binomial confidence intervals.

Fig. 4.2: Phototoxic effects (impaired or dead) of dissolved oil on copepods in experiment 2 (*N. flemingeri* and *N. plumchrus*; N= 15 -19, pooled data; top panel) and total PAC concentrations in the exposure water at the start and end of the oil exposure (bottom panel). Bars indicate binomial confidence intervals.

Fig. 4.3: Correlations of phototoxicity, PAC concentration and light dose. Regression of phototoxic effect in Full spectrum sunlight (left panel) and in sunlight with UVB - exclusion (right panel) and, 1) PAC concentration in the exposure water (a, b), 2) the product of PAC concentration in the exposure water with the exposure light dose (c, d), and 3) the product of PAC concentration in copepod tissue and the exposure light dose (experiment 1 only) (e, f). ▲ = experiment 1; ■ = experiment 2.

Fig. 4.4: Comparison of total light intensities measured on a clear day (May 11, 01) and a day with intermittent disk visibility, measured during experiment 2 (June 5, 01) in Seward, Alaska. All measurements were taken under 10 cm water column.

Table 4.1: Total light doses and ranges of light intensity of visible light (RFF), UVA and UVB measured during the two experiments.

Exp.		RFF		UVA		UVB	
	Exposure Duration [min]	Total Dose [W * m^{-2} * min^{-1}]	Range Intensity [W * m^{-2}]	Total Dose [W * m^{-2} * min^{-1}]	Range Intensity [W * m^{-2}]	Total Dose [W * m^{-2} * min^{-1}]	Range Intensity [W * m^{-2}]
1	230 (day 1)	27400	21-216	4508	4.2-30	113	0.1-0.8
	200 (day 2)	36438	103-322	5096	15.8-42.2	138	0.4-1.0
	430 (total)	63838	21-322	9604	4.2-42.2	251	0.1-1.0
2	280	44812	102-241	7027	16-36	212	0.5-1.1

Figure 4.1: Phototoxic effects of dissolved oil on copepods and total PAC concentrations in the exposure water (experiment 1).

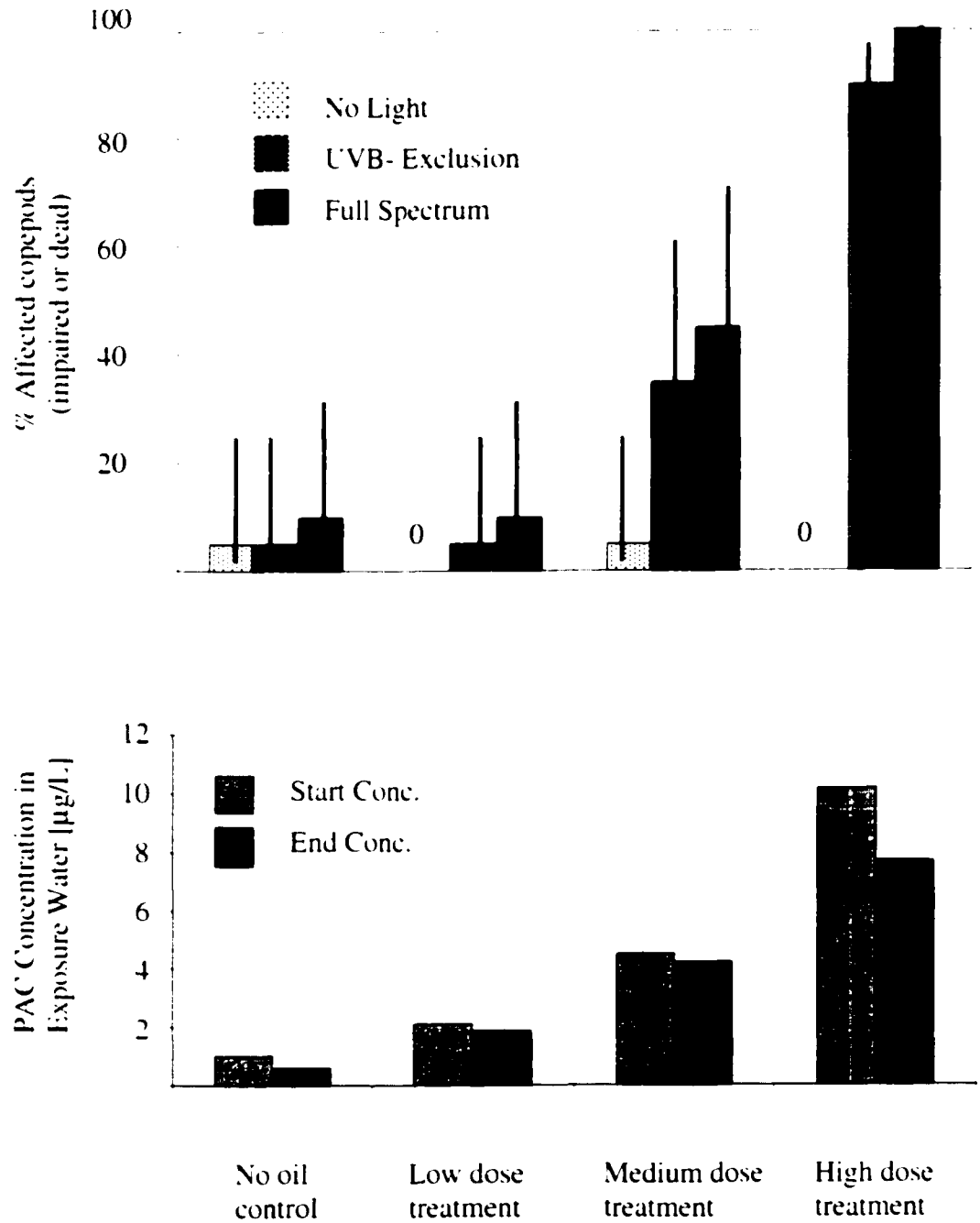


Figure 4.2: Phototoxic effects of dissolved oil on copepods and total PAC concentrations in the exposure water (experiment 2).

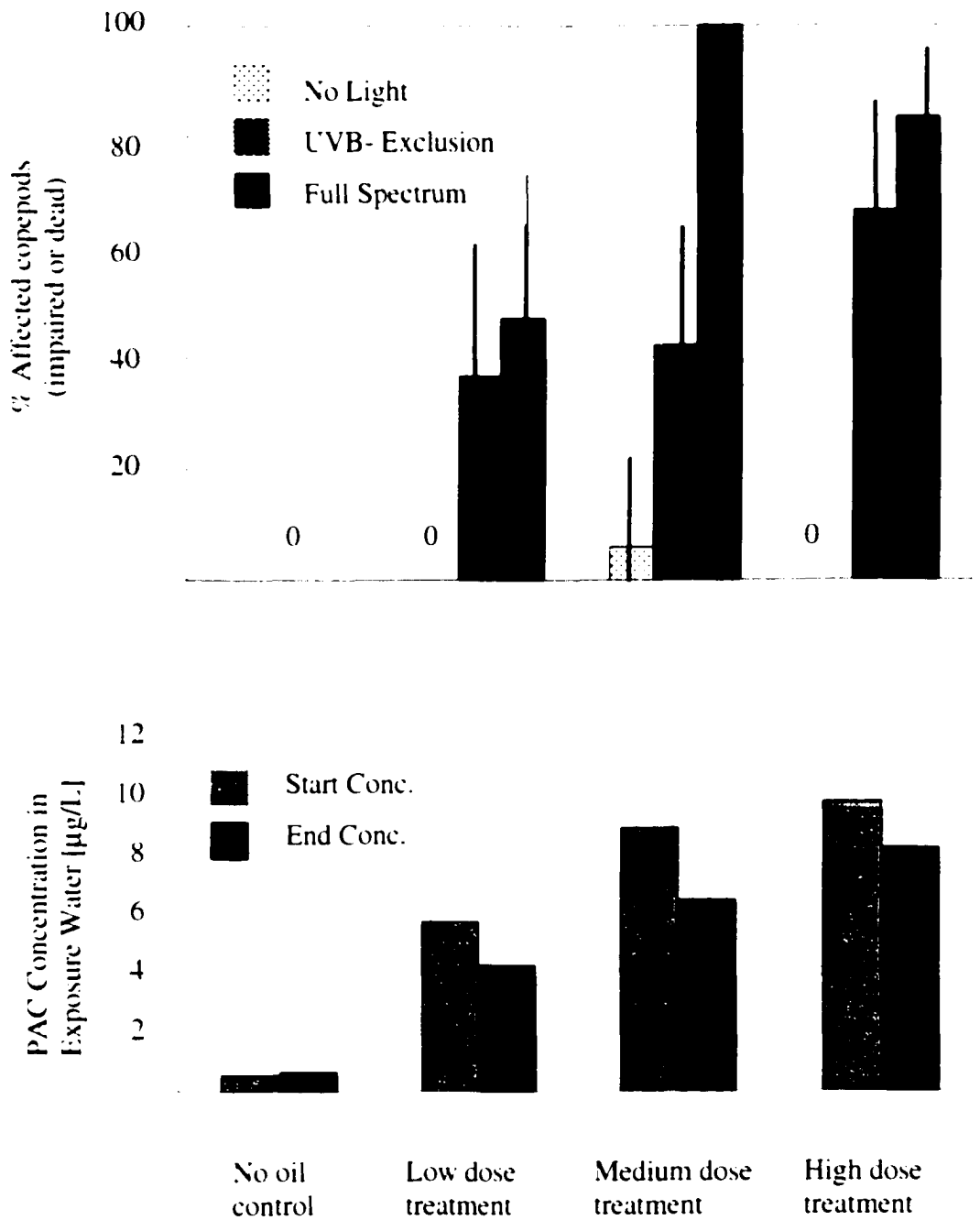


Figure 4.3: Correlations of phototoxicity, PAC concentration and light dose.

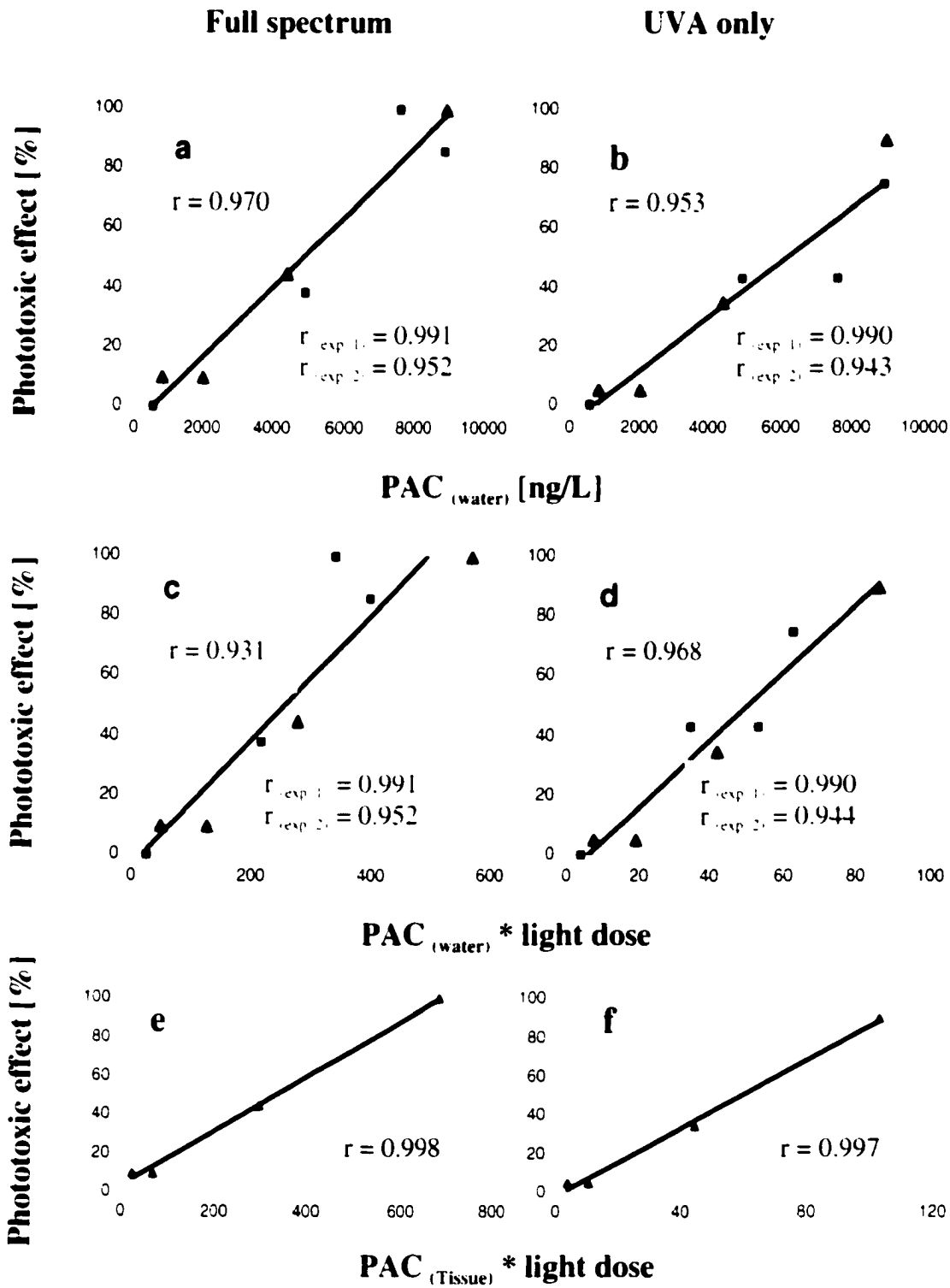
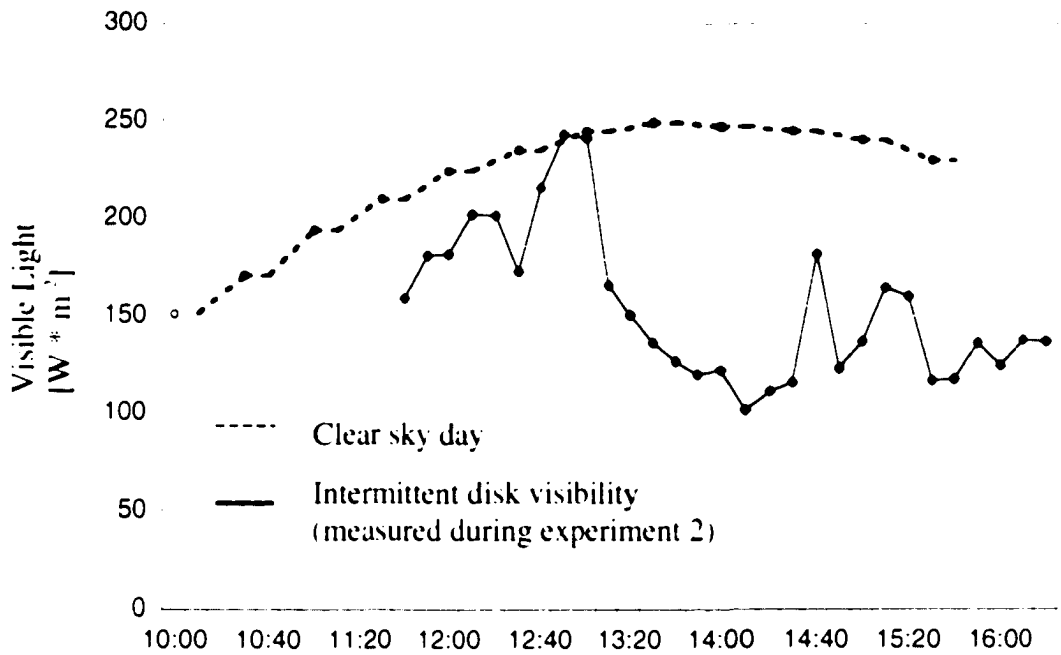


Figure 4.4: Comparison of total light intensities measured on a clear day and a day with intermittent disk visibility.



Chapter 5

EGG PRODUCTION OF STARVED *CALANUS MARSHALLAE* AND *PSEUDOCALANUS SPP.* AND THE POTENTIAL FOR THE DISTRIBUTION OF POLYAROMATIC COMPOUNDS IN COPEPOD EGGS

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ABSTRACT

This study investigated the feasibility of culture experiments to compare egg production rates and survival of oiled and unoiled female *Calanus marshallae* and *Pseudocalanus* spp. copepods. Twenty *C. marshallae* were cultured and egg production and mortality were monitored over 28 days. Sixty *Pseudocalanus* spp. were maintained in culture wells and egg production and mortality were monitored over four days. Copepods in cultures were unoiled and not fed. *C. marshallae* had little mortality for the first two weeks; after four weeks in culture 50 % survived. Spawning activity and mean egg production declined rapidly after the third day in culture. In *Pseudocalanus* spp. mortality increased from 5 % on the first day in culture to 36 % on day four. Accordingly, spawning activity and egg production declined with each subsequent day. Egg production data from these experiments were used to calculate a sample size for determining a significant difference in mean egg production between two treatments. For *C. marshallae* the experimental design was rejected based on high natural variability in egg production and the difficulty in obtaining large numbers of gravid females. High mortality and strong decline in egg production early in the experiment made *Pseudocalanus* spp. unsuited for the intended comparison of egg production rates between previously oiled and unoiled females, because of the duration of the oil exposure (24 hours prior to the start of the incubation for egg counts). However, mortality increased more rapidly in 11 oiled female *C. marshallae* than in unoiled females after 19 days of starvation.

INTRODUCTION

Eggs of previously oiled female copepods may receive polyaromatic compounds (PAC) when lipids are incorporated into egg tissue. Copepods accumulate, store and release PAC when exposed to the water soluble fraction of oil (Lee 1975; Duesterloh et al., 2002). Because of the lipophilic properties of PAC, uptake has been suspected to be associated with lipid stores in copepods (Corner 1975). The seasonal lipid stores of large copepods in higher latitudes are mainly used for gonad development and egg production (Evanson et al., 2000; Hagen and Schnack-Schiel, 1996; Sargent and Falk-Petersen, 1988). High lipid contents of eggs were reported for *Euchaeta japonica* and *Calanus pacificus* (Sargent and Falk-Petersen, 1988).

If toxic PAC are passed from females to eggs, effects on viability, hatching rates and survival of offspring may occur. Reductions in reproduction rates in response to exposure to anthracene were observed with the water flea *Daphnia magna* (Holst and Giesy, 1989). Depressed copepod populations may negatively affect survival of higher trophic level consumers like fish, birds and marine mammals and influence the length and productivity of the phytoplankton bloom and sedimentation rates (Parsons and Lalli, 1988). Conversely, concentration and composition of the phytoplankton can influence copepod production rates (Ban et al., 1997; Frost 1985; Peterson 1988). Also, vertically migrating copepods carrying PAC may introduce these toxic compounds at depth when spawning. Differences in life history patterns and the number of generations produced per year determine the timing and depth of egg release. For example, *Pseudocalanus* spp. have

continuous egg production throughout 6 - 7 months of the year while they are actively feeding in surface water (0 - 50 m) (Frost 1985; Paul et al., 1990). In contrast, spawning of *Neocalanus plumchrus* is limited from January to mid April at depths below 300 m and is entirely dependent on internal lipid stores (Fulton 1973; Evanson et al., 2000).

Toxic effects of oil may become evident only at times of mobilization of storage lipids. Thus, toxicity of oil to copepods may be underestimated in short term toxicity studies. Lethal concentrations (LD_{50}) of oil to copepods vary widely from 1 to 1350 µg/Liter and are higher than for most other plankton taxa tested (Capuzzo 1987; Lee 1977). Some of the variation is due to differences in oil composition and test conditions. However, female *Eurytemora affinis* treated with ^{14}C-labeled naphthalene retained about 10 % of the radioactivity originally taken up and had by 25 % reduced egg production and life span (Corner 1975). Feasibility of a comparison of egg production of oiled and unoiled females of *Calanus marshallae* and *Pseudocalanus* spp. was tested in this study by evaluating the underlying natural variability in egg production and survival in cultures under conditions of food limitation. A comparison of survival of oiled and unoiled female *C. marshallae* is presented and implications of delayed oil toxicity to copepods are discussed in this article.

METHODS

Zooplankton Collection

Calanus marshallae samples were collected on July 25, 2000 in Lynn Canal, southeastern Alaska, with a 330 µm mesh open ring plankton net towed vertically from a maximum depth of 100 m. *Pseudocalanus* spp. were collected in Auke Bay on August 25, 2000 with a 155 µm mesh open ring plankton net towed vertically from a maximum depth of 20 m.

Egg production experiments

Females of *C. marshallae* and *Pseudocalanus* spp. were identified by microscopic examination and pipetted individually into test chambers containing filtered seawater.

Twenty *C. marshallae* were cultured in glass beakers with approximately 50 ml seawater in a constant-temperature walk-in chamber set at 6 °C. Egg production was monitored on days 1, 3, 4, 6, 8, 10, 13, 15, 17, 19, 24 and 28 by microscopic examination. If eggs were present, they were counted and removed. The water in the test chambers was partially exchanged at every examination and completely exchanged twice weekly.

Sixty *Pseudocalanus* spp. were cultured in 1 ml culture wells and maintained in a flow through water bath at ambient Auke Bay water temperature (10 - 12°C). Monitoring for survival and egg production was conducted microscopically every 24 hours. At this time, eggs were removed and the water in the test chambers was partially exchanged.

Egg counts of *C. marshallae* were used to estimate a sample size that would be sufficient to detect differences in egg production between the means of two treatments (oiled and unoiled). Egg production values are presented as mean ± 1 standard deviation. The experiment was assumed to resemble a simple random sample of spawning events in the natural population, and the number of egg clutches in the population was assumed large. The sample variance was calculated from all clutches in the experiment and used as an unbiased estimator for the population variance. Confidence limits were chosen at 0.95 and the maximum allowable difference *d* between the estimate and the true value was chosen to be 1.7 eggs, resembling approximately 10 % of the maximum mean egg production in this experiment (Thompson 1992).

Post-oil survival experiment

Eleven female *C. marshallae* were exposed to a low dose preparation (total PAC of ~2 µg/Liter) of the water soluble fraction of weathered Alaska North Slope crude oil for 24 hours. The flow-through exposure system and PAC composition of the exposure water were reported (Duesterloh et al., 2002). The exposure water contained primarily three and four ring PAC including naphthalenes, flourenes, dibenzothiophenes, phenathrenes and trace concentrations of chrysenes. Following the oil exposure, females were sorted into individual beakers containing ~50 ml of filtered seawater and maintained at 6 °C. As a control, 11 females that had not been treated with the oil preparation were cultured in the same way. Survival and egg production were checked daily until death; water was exchanged approximately every 48 hours.

RESULTS

Egg production experiments

C. marshallae had less than 10 % mortality for the first two weeks. In the third and fourth week mortality increased, but after four weeks in culture 50 % of the initial females were still alive (Figure 1a). Egg clutches contained between one and 54 eggs. For most females only one spawning event was observed but three females laid eggs twice during the experiment. The highest spawning activity was measured on day three (over 50 % of all females), then spawning activity dropped to < 5 - 15 % until day 13, when spawning ceased entirely. The time of cessation of spawning activity was concurrent with an increase in mortality (Figure 1b). Mean daily egg production varied from 17.6 (± 18.5) on day three to 0.05 (± 0.2) on day 10 (all females included) (Figure 1c).

Over the course of the experiment with 20 females a total of 22 egg clutches were recorded; four females did not spawn. Sample variance was calculated and used as an unbiased estimator for the population variance. With

$\hat{\sigma}^2 = s^2 = 242,$

and the assumption that N =∞, equation (1) (Thompson, 1992) resolves to 138 egg clutches. In this equation n is the sample size, d is the maximum allowable difference between the estimate and the true value, and z denotes the upper $\alpha/2$ point of the standard normal distribution.

$$n = \frac{1}{\left(\frac{d}{\sigma}\right) + \frac{1}{t}}$$

(1)

Consequently, 138 egg clutches are needed per treatment to determine the mean daily egg production within the allowable range defined by d (± 1.7) with 95 % confidence. Since 20 females produced 22 egg clutches, 126 females are needed to produce 138 egg clutches and 252 females would be needed for the two treatments (oiled and unoiled) of the experiment. Limited availability of females in the study area precluded an experiment of that magnitude.

Pseudocalanus spp. had 5 % mortality on day 1, 12.3 %, 18 % and 36.6 % on days two, three and four, respectively (Figure 1d). Fecundity was between one and 16 eggs per day per female and females were observed to lay eggs on subsequent days. Mean egg production over all was 2.8 (± 3.9) on day one, 0.8 (± 1.7) and 0.1 (± 0.5) on days two and three, respectively, and dropped to zero on day 4 (Figure 5). If only producing females were included in the calculation, mean egg production was 4.5 (± 4.1) eggs per female per day on day one, 2.9 (± 2.4) on day two, and 2 (± 1) on day three (Figure 1f). Over 60 % of females were spawning initially, but spawning activity dropped to 28 % and less than 10 % on days 2 and 3, respectively (Figure 1e).

Post-oil survival experiment

For the first 17 days in culture mortality in the oiled and unoiled treatments was low (1 female died in each treatment). Between day 18 and day 37 the mortality rate [%] in the oiled treatment was twice as high as in the unoiled treatment. On day 37 the experiment was terminated with a total of 10 deaths in the oiled treatment and 7 deaths in the unoiled treatment (Figure 2).

DISCUSSION

Mean egg production in *C. marshallae* in this study was lower than reported from laboratory cultured females fed excess amounts of *Thalassiosira weissflogii* (Peterson 1988), but spawning continued for 14 days without food. The range of clutch sizes (1 - 54 eggs) coincides with the reported range of 1 - 61 eggs per clutch (Peterson 1988). However, the highest mean daily egg production of 17.6 (± 18.5) measured in this study is approximately half of the values reported for the months of June to August (31.3 - 32.2 eggs per clutch) (Peterson 1988). Cessation of egg production in starved females was observed 24 hours after the start of the incubation without food (Peterson 1988). Inconsistent with this finding, I observed the highest egg production during the 48 hour period between the first and third day in culture, and egg production continued at low rates until day 14. These differences in mean egg production and reproductive timing may be in part caused by temperature differences; incubations were at 6°C in this study and at 10°C in the former. The size of egg clutches and the interval between clutches vary seasonally and between latitudes within species of *Calanus* (Mauchline 1998). Based

only on the variance observed in this experiment, a sample size to detect a difference in mean egg production between two treatments was estimated. Most *C. marshallae* in the net samples were stage V copepodites and females were rare in comparison. Thus, it was not feasible to conduct an experiment comparing effects of oil on egg production with this species.

The genus *Pseudocalanus* contains at least seven distinct species, four of which (*P. minutus*, *P. moultoni*, *P. newmani*, *P. mimus*) may occur in southeast Alaska (Frost 1989). Morphological differences between the species are slight and no attempt was made in this study to identify *Pseudocalanus* beyond genus. Mean egg production rates in *Pseudocalanus* spp. were studied in Auke Bay in 1987 and 1988 in 24-hour egg production assessments (Paul et al., 1990). Thus, data gathered on day 1 in this study were directly comparable to the previous study. Egg production in this study was slightly higher when egg-producing and non-producing females were considered, but standard deviation was also higher (2.86 ± 3.9 and 2.6 ± 1.1). If non-producing females were not included in the calculation, mean egg production in this study was lower (4.53 ± 4.1) than reported for 1987 (7.4 ± 3.7) and 1988 (8.3 ± 4.5) (Paul et al., 1990). Female *Pseudocalanus* spp. in this study in June 2000 produced fewer eggs than in April and May, 1987 and 1988 (Paul et al., 1990), but the percentage of spawners was higher (~ 60 %). This is consistent with a proposed decline in egg production per female toward the end of a season due to aging, and a steady increase in the percentage of active spawners toward the later season (Paul et al., 1990).

Food availability is the principal factor influencing egg production rates in copepods (Mauchline 1998). High mortality and strong decline in egg production early in the experiment made *Pseudocalanus* spp. unsuited for the intended comparison of egg production rates between previously oiled and unoiled females, because of the duration of the oil exposure (24 hours prior to the start of the incubation for egg counts). The high mortality in the incubations with *Pseudocalanus* spp. was assumed to be an effect of starvation. Generally, egg production increases as food concentration increases to achieve an asymptotic level (Mauchline 1998). However, egg production may be affected by food availability in some species and not in others; phytoplankton bloom conditions accelerated egg production in *Calanus pacificus* but not in the co-occurring *Pseudocalanus* sp. in Dabob Bay, Washington (Frost 1985). Inconsistent with my observation of high starvation mortality and reduction in egg production, *Pseudocalanus elongatus* females incubated in filtered seawater resumed egg production for up to 6 days (Frost 1985). I conclude that starvation may have acted in synergism with another unidentified stress factor to cause the responses observed in these experiments with *Pseudocalanus* spp.

Low dosage oil exposure may adversely affect long term survival in copepods. Consistent with the reduced life span in oiled *Eurytemora affinis* (Corner 1975), mortality in previously oil exposed *C. marshallae* females in this study occurred about three days sooner and at a higher rate then in unoiled females. In the current experiment egg production rates were not compared because of the small sample size. *Calanus*

marshallae has previously been reported to have no immediate mortality in response to low dosage oil treatments as used in this experiment (Duesterloh et al., 2002). However, due to their high lipid content these copepods readily bioaccumulate PAC from aqueous solution (accumulation factor ~8000). Although no data were collected on long term retention of PAC in this study, a 10 % retention of naphthalene in female *Eurytemora affinis* and retention of PAC by nauplii lasting throughout several life stages has been reported (Corner 1975). Like many high latitude copepod species, late stages of *C. marshallae* accumulate a lipid store for gonad development, egg production and, to a lesser extent, metabolic needs during the winter months (Evanson et al., 2000; Sargent and Falk-Petersen, 1988). Because of the lipophilic properties of PAC the majority of retained PAC may be assumed to be associated with the seasonal lipid stores in the lipid sac. I speculate that toxic PAC are not harmful to the copepods as long as they are stored in a metabolically inactive form. However, at times of metabolic activation of the lipid stores, the toxic PAC may be reactivated and cause reduction in reproductive output and premature mortality.

Bioaccumulation and retention of toxic PAC and a reduction of reproductive success in copepods may impact trophic interactions in marine communities. Copepods concentrate PAC from a dilute solution in their environment and deposit them in their lipid stores, thus making them accessible to higher trophic level predators. Copepods are prey to many planktivores including forage fish and the juveniles of numerous commercial species (e.g., salmon, pollock, herring) as well as some invertebrates. Feeding on oil

contaminated prey by pink salmon fry has been reported to reduce growth rates (Carls et al., 1996). More research is necessary to assess the magnitude and possible impact of this transport mechanism on natural fish populations.

Depressed copepod populations resulting from decreased reproduction rates in response to oil exposure may interrupt the energy transfer from primary production to higher trophic level consumers. Copepods form an important link in the food chain by transforming primary production from phytoplankton into animal protein, accessible to predators such as fish. Exposure of several copepod species to industrial waste discharge resulted in reductions in feeding rates, respiration rates and production rates. Toxic waste exposure was energetically equivalent to food limitation (Capuzzo 1985). Total egg production of a population, and other variables determine the population size of the next generation. Low copepod abundance resulting from reduced reproductive success may cause food limitation in those predators with limited mobility (e.g., larval fish), while mobile predators disperse in search of better food resources. The dependence of larval fish recruitment on zooplankton stocks was demonstrated in Auke Bay, Alaska (Haldorson et al., 1993; Coyle and Paul, 1992). In *Pseudocalanus* spp. female abundance at the time of the onset of the spring phytoplankton bloom was a more important factor for nauplii production and larval fish recruitment than the strength of the phytoplankton bloom (Paul et al., 1990).

The proposed association of PAC with the lipid stores of copepods suggests that when lipid stores are utilized for egg production, PAC are incorporated in egg tissue. Ontogenetic vertical migration in copepods may transport PAC contained in egg tissue to depth, possibly introducing them into pelagic and benthic food chains. The synthesis of storage lipids from non-lipid dietary precursors and the utilization of lipids for gonad development and egg production was studied in detail for the copepods *C. finmarchicus* and *Metridia longa* (Sargent and Falk-Petersen, 1988). Oil droplets were observed in freshly laid eggs of *C. pacificus* (Fulton 1973). *C. pacificus* is taxonomically very similar to *C. marshallae* but females have reduced mouth parts and do not feed, so that egg production is entirely dependent upon the lipid stores, whereas in *C. marshallae* egg production is dependent on food availability (Fulton 1973; Peterson 1988). This suggests that *C. marshallae* lay eggs within the upper 50 m of the water column, which is their spring and summer habitat, while *C. pacificus* spawn at depths below 300 m (Fulton 1973). The life cycles of the larger species of the genus *Neocalanus*, which dominate the biomass in high latitude offshore plankton communities (Cooney 1986) are generally similar to that of *C. pacificus* in that only one generation is produced per year and spawning occurs at depth during the winter months (Miller and Clemons, 1988; Miller 1993). Vertical transport of PAC in eggs thus depends on the predominant species of copepods and their life cycle, and may be of importance in some regions but not in others.

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Figure captions

Fig. 5.1: Comparison of mortality, spawning activity and mean egg production in females of 20 *Calanus marshallae* and 60 *Pseudocalanus* spp. cultured without food for 28 (*C. marshallae*) and 4 (*Pseudocalanus* spp.) days. Standard deviations of mean egg production, which are referred to in the text are given in parentheses.

Fig. 5.2: Mortality rates of 11 oiled and 11 unoiled *Calanus marshallae* females in cultures without food. The 24 hr oil exposure occurred on day 1 at a concentration of ~2 µg/L total PAC.

Figure 5.1: Comparison of mortality, spawning activity and mean egg production.

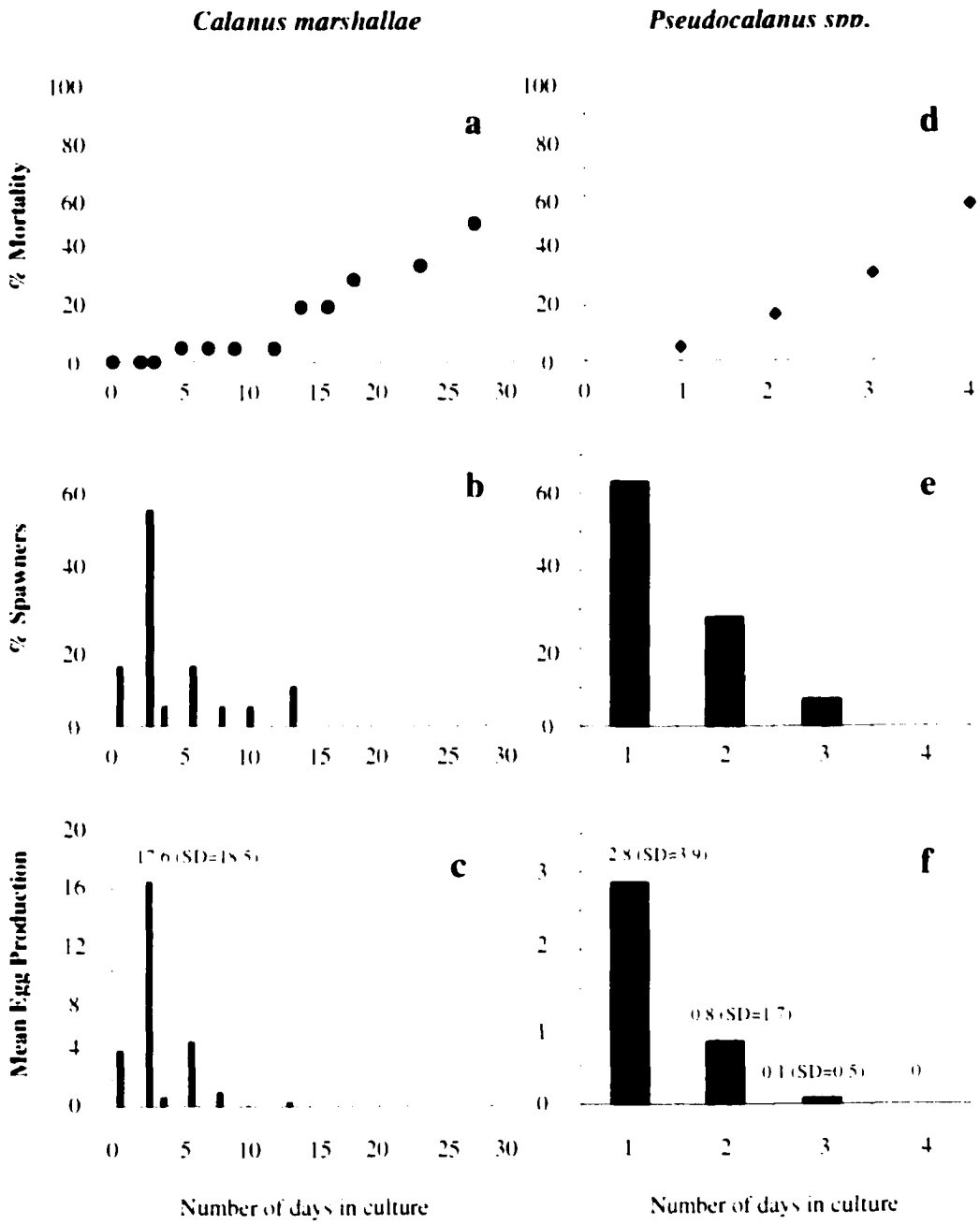
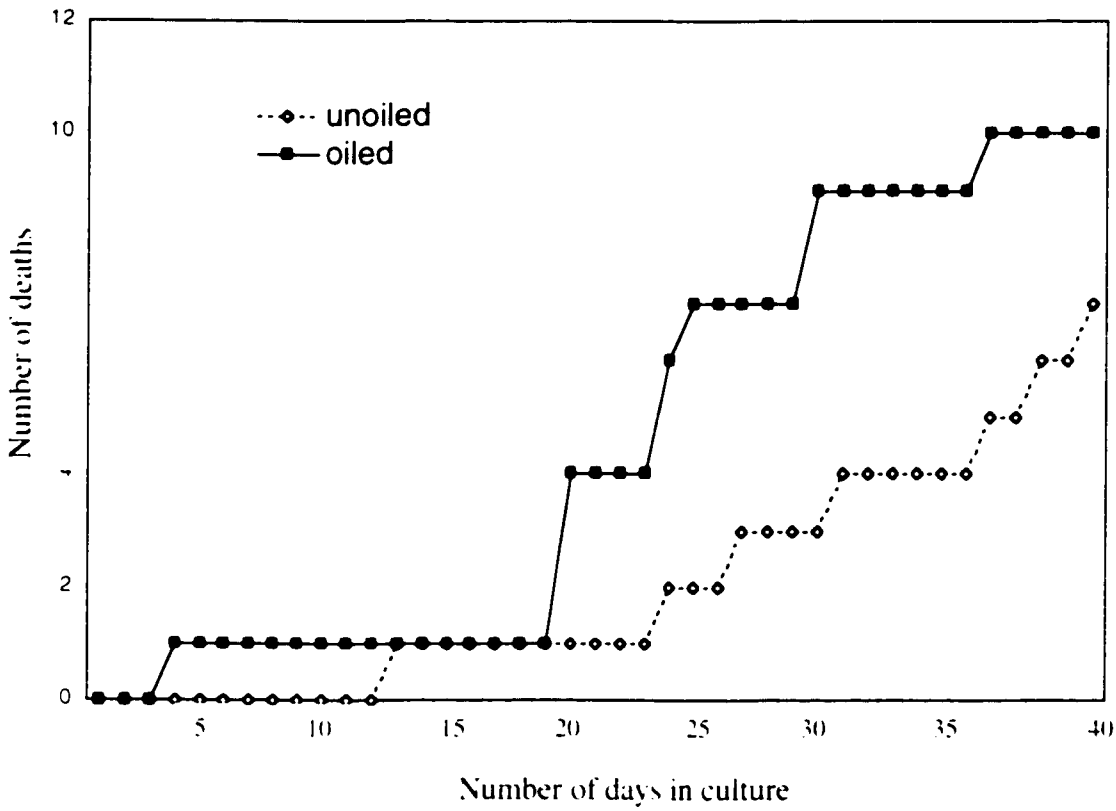


Figure 5.2: Mortality rates of 11 oiled and 11 unoiled *Calanus marshallae* females.



Appendix 1. Sample codes, matrix and sampling information. Original Chain of custody sheets. REF = reference sample (fixed in ethanol); LIP = lipid sample; REP or R = replicate; W = water; TIS or T = tissue; low, l, medium, high, h, control, c, highhigh, dose all refer to various PAC concentrations in the exposure water; T0, T24 = start and end time of oil exposure, respectively; ABL = Auke Bay Laboratory; PWS = Prince William Sound; Lab = Institute of Marine Science Laboratory, Seward; GAK 1 = Gulf of Alaska Station 1; GOA = Gulf of Alaska; NC or Neocal. = Neocalanus; N. flem. or flem = *Neocalanus flemingeri*, N. plum. or plum = *Neocalanus plumchrus*; All tissue samples were copepodite stage V, this is sometimes noted as CV.

Chain of custody Form **Serial # 11063**

Assigned Sample #	Collector's Sample code	Date Collected	Matrix &Species, etc	Location Collected	Method	etc
- 01	01P4-W	071200	Water	ABL		
- 02	02P4-W	071201	Water	ABL		
- 03	03P4-W	071202	Water	ABL		
- 04	04P4-W	071203	Water	ABL		
- 05	05P4-W	071204	Water	ABL		
- 06	06P4-W	071205	Water	ABL		
- 07	07P4-W	071206	Water	ABL		
- 08	08P4-W	071207	Water	ABL		
- 09	09P4-T	071208	Tissue	ABL		
- 10	10P4-T	071209	Tissue	ABL		
- 11	11P4-T	071210	Tissue	ABL		
- 12	12P4-T	071211	Tissue	ABL		
- 13	01P5-W	071800	Water	ABL		
- 14	02P5-W	071801	Water	ABL		
- 15	03P5-W	071802	Water	ABL		
- 16	04P5-W	071803	Water	ABL		
- 17	05P5-W	071804	Water	ABL		
- 18	06P5-W	071805	Water	ABL		
- 19	07P5-W	071806	Water	ABL		
- 20	08P5-W	071807	Water	ABL		
- 21	09P5-T	071808	Tissue	ABL		
- 22	10P5-T	071809	Tissue	ABL		
- 23	11P5-T	071810	Tissue	ABL		
- 24	12P5-T	071811	Tissue	ABL		
- 25	13P5-T	071812	Tissue	ABL		
- 26	01P6-W	081100	Water	ABL		
- 27	02P6-W	081101	Water	ABL		
- 28	03P6-W	081102	Water	ABL		
- 29	04P6-W	081103	Water	ABL		
- 30	05P6-W(T24)	081104	Water	ABL		
- 31	06P6-T(T24)	081105	Tissue	ABL		

Appendix 1 (cont.).

- 32	07P6-T(T24)	081106	Tissue	ABL
- 33	08P6-T(T24)	081107	Tissue	ABL
- 34	09P6-T(T48)	081108	Tissue	ABL
- 35	10P6-T(T48)	081109	Tissue	ABL
- 36	11P6-T(T48)	081110	Tissue	ABL
- 37	12P6-W(T48)	081111	Water	ABL
- 38	13P6-T(T96)	081112	Tissue	ABL
- 39	14P6-T(T96)	081113	Tissue	ABL
- 40	15P6-T(T96)	081114	Tissue	ABL
- 41	16P6-T(T96)	081115	Tissue	ABL
- 42	17P6-W(T96)	081116	Water	ABL
- 43	18P6-W(T96)	081117	Water	ABL
- 44	19P6(b)-W(T96)	081118	Water	ABL
- 45	20P6(b)-W(T96)	081119	Water	ABL
- 46	21P6(b)-T(T96)	081120	Tissue	ABL
- 47	22P6(b)-T(T96)	081121	Tissue	ABL

Chain of custody Form **Serial # 11064**

Assigned Sample #	Collector's Sample code	Date Collected	Matrix &Species, etc	Location Collected	Method	etc
- 01	REF 1.1	041601	20 Neocal. CV	PWS	open ring	ETOH
- 02	LIP 1 1 REP 1	041601	10 Neocal. CV	PWS	200µ	frozen
- 03	LIP 1 1 REP 2	041601	10 Neocal. CV	PWS	"	frozen
- 04	LIP 1 1 REP 3	041601	10 Neocal. CV	PWS	"	frozen
- 05	W 1 1 low	041801	water T0	Lab	900ml	
- 06	W 1 1 high	041801	water T0	Lab	900ml	
- 07	W 1 1 control	041801	water T0	Lab	900ml	
- 08	W 1 1 low	041901	water T24	Lab	900ml	
- 09	W 1 1 high	041901	water T24	Lab	900ml	
- 10	W 1.1 control	041901	water T24	Lab	900ml	
- 11	TIS 1.1R1low	041901	10 NC	PWS	frozen	
- 12	TIS 1.1R2low	041901	10 NC	PWS	frozen	
- 13	TIS 1 1R3low	041901	9 NC	PWS	frozen	
- 14	TIS 1.1R1high	042101	10 NC	PWS	frozen	
- 15	TIS 1.1R2high	041901	10 NC	PWS	frozen	
- 16	TIS 1.1R3high	041901	10 NC	PWS	frozen	
- 17	TIS 1.1R1con	041901	6 NC	PWS	frozen	
- 18	TIS 1.1R2con	041901	10 NC	PWS	frozen	
- 19	TIS 1.1R3con	041901	10 NC	PWS	frozen	
- 20	W 2.1 high	042001	water T0	Lab	900ml	
- 21	W 2.1 highhigh	042001	water T0	Lab	900ml	
- 22	W 2.1 control	042001	water T0	Lab	900ml	
- 23	W 2 1 high	042101	water T24	Lab	900ml	
- 24	W 2.1 highhigh	042101	water T24	Lab	900ml	
- 25	W 2.1 control	042101	water T24	Lab	900ml	
- 26	REF 1.2	050101	20 NC	GAK1	ETOH	
- 27	LIP 1.2REP1	050101	10 NC	GAK1	frozen	

Appendix 1 (cont.).

- 28	LIP 1.2REP2	050101	10 NC	GAK1	frozen
- 29	LIP 1.2REP3	050101	10 NC	GAK1	frozen
- 30	REF 1.3	050201	20 NC	PWS	ETOH
- 31	LIP 1.3REP1	050201	10 NC	PWS	frozen
- 32	LIP 1.3REP2	050201	10 NC	PWS	frozen
- 33	LIP 1.3REP3	050201	10 NC	PWS	frozen
- 34	W 1.2 control	050201	water T0	Lab	900ml
- 35	W 1 2 low	050201	water T0	Lab	900ml
- 36	W 1.2 high	050201	water T0	Lab	900ml
- 37	1.3DW REP1	050201	10 NC	PWS	frozen
- 38	1.3DW REP2	050201	10 NC	PWS	frozen
- 39	1 3DW REP3	050201	10 NC	PWS	frozen
- 40	1.2DW REP1	050301	10 NC	GOA	frozen
- 41	1 2DW REP2	050301	10 NC	GOA	frozen
- 42	1.2DW REP3	050301	10 NC	GOA	frozen
- 43	W 1.2 control	050301	water T24	Lab	900ml
- 44	W 1.2 low	050301	water T24	Lab	900ml
- 45	W 1.2 high	050301	water T24	Lab	900ml
- 46	T 1.2 R1 c	050301	10 NC	GOA	frozen
- 47	T 1.2 R2 c	050301	10 NC	GOA	frozen
- 48	T 1.2 R3 c	050301	10 NC	GOA	frozen
- 49	T 1.2 R1 l	050301	10 NC	GOA	frozen
- 50	T 1.2 R2 l	050301	10 NC	GOA	frozen

Chain of custody Form **Serial # 12009**

Assigned Sample #	Collector's Sample code	Date Collected	Matrix &Species, etc	Location Collected	Method	etc
- 01	T 1.2 R3l	050301	10 NC CV	GOA	frozen	
- 02	T 1 2 R1h	050301	10 NC CV	GOA	frozen	
- 03	T 1 2 R2h	050301	10 NC CV	GOA	frozen	
- 04	T 1 2 R3h	050301	10 NC CV	GOA	frozen	
- 05	W 1 3 control	050401	water T24	Lab	900ml	
- 06	W 1.3 low	050401	water T24	Lab	900ml	
- 07	W 1.3 high	050401	water T24	Lab	900ml	
- 08	T 1.3 R1 c	050401	10 NC CV	PWS	frozen	
- 09	T 1 3 R2 c	050401	10 NC CV	PWS	frozen	
- 10	T 1.3 R3 c	050401	10 NC CV	PWS	frozen	
- 11	T 1.3 R1 l	050401	10 NC CV	PWS	frozen	
- 12	T 1.3 R2 l	050401	10 NC CV	PWS	frozen	
- 13	T 1.3 R3 l	050401	10 NC CV	PWS	frozen	
- 14	T 1.3 R1 h	050401	10 NC CV	PWS	frozen	
- 15	T 1.3 R2 h	050401	10 NC CV	PWS	frozen	
- 16	T 1.3 R3 h	050401	10 NC CV	PWS	frozen	
- 17	LIP 3.1 R1	051401	10 NC CV	Cape Clear	frozen	
- 18	LIP 3.1 R2	051401	10 NC CV	Cape Clear	frozen	
- 19	LIP 3.1 R3	051401	10 NC CV	Cape Clear	frozen	
- 20	REF 3.1	051401	20 NC CV	Cape Clear	ETOH	
- 21	DW 3.1 R1	051401	10 N. flem.	Cape Clear	frozen	
- 22	LIP 3.1 R1 Np	051401	10 N. plum.	Cape Clear	frozen	
- 23	LIP 3.1 R2 Np	051401	10 N. plum.	Cape Clear	frozen	
- 24	LIP 3.1 R3 Np	051401	10 N. plum.	Cape Clear	frozen	

Appendix 1 (cont.).

- 25	W 3.1 control	051501	water T0	Lab	900ml
- 26	W 3.1 dose 2	051501	water T0	Lab	900ml
- 27	W 3.1 dose 3	051501	water T0	Lab	900ml
- 28	W 3.1 dose 4	051501	water T0	Lab	900ml
- 29	W 3.1 control	051601	water T24	Lab	900ml
- 30	W 3.1 dose 2	051601	water T24	Lab	900ml
- 31	W 3.1 dose 3	051601	water T24	Lab	900ml
- 32	W 3.1 dose 4	051601	water T24	Lab	900ml
- 33	T 3.1 R1 c	051601	10 NC CV	Cape Clear	frozen
- 34	T 3.1 R2 c	051601	10 NC CV	Cape Clear	frozen
- 35	T 3.1 R3 c	051601	10 NC CV	Cape Clear	frozen
- 36	T 3.1 R1dose 2	051601	10 NC CV	Cape Clear	frozen
- 37	T 3.1 R2dose 2	051601	10 NC CV	Cape Clear	frozen
- 38	T 3.1 R3dose 2	051601	10 NC CV	Cape Clear	frozen
- 39	T 3.1 R1dose 3	051601	10 NC CV	Cape Clear	frozen
- 40	T 3.1 R2dose 3	051601	10 NC CV	Cape Clear	frozen
- 41	T 3.1 R3dose 3	051601	10 NC CV	Cape Clear	frozen
- 42	T 3.1 R1dose 4	051601	10 NC CV	Cape Clear	frozen
- 43	T 3.1 R2dose 4	051601	10 NC CV	Cape Clear	frozen
- 44	T 3.1 R3dose 4	051601	10 NC CV	Cape Clear	frozen
- 45	DW 3.1 R2	051601	10 NC CV	Cape Clear	frozen
- 46	DW 3.1 R3	051601	10 NC CV	Cape Clear	frozen
- 47	T 3.1 R4dose 3	051601	10 NC CV	Cape Clear	frozen
- 48	T 3.1 R4dose 4	051601	10 NC CV	Cape Clear	frozen
- 49	W 2.2 control	051801	water T0	Lab	900ml
- 50	W 2.2high dose	051801	water T0	Lab	900ml

Chain of custody Form **Serial # 12032**

Assigned Sample #	Collector's Sample code	Date Collected	Matrix &Species. etc	Location Collected	Method	etc
- 01	W2.2 control	051901	water T24	Lab	900 mL	
- 02	W2.2 high	051901	water T24	Lab	900 mL	
- 03	W5.1 control	052201	Water T0	Lab	900 mL	exp. stopped
- 04	W5.1 copis	052201	Water T0	Lab	900 mL	exp. stopped
- 05	W5.1 algae	052201	Water T0	Lab	900 mL	exp. stopped
- 06	W1.4 control	060101	Water T0	Lab	900 mL	
- 07	W1.4 low	060101	Water T0	Lab	900 mL	
- 08	W1.4 high	060101	Water T0	Lab	900 mL	
- 09	REF 1.4	060101	10 flem+10 plum	GAK 1	ETOH	
- 10	LIP 1.4 Rep 1.1	060101	10 flem	GAK 1	frozen	
- 11	LIP 1.4 Rep 2.1	060101	10 flem	GAK 1	frozen	
- 12	LIP 1.4 Rep 3.1	060101	10 flem	GAK 1	frozen	
- 13	LIP 1.4 Rep 1.2	060101	10 plum	GAK 1	frozen	
- 14	LIP 1.4 Rep 2.2	060101	10 plum	GAK 1	frozen	
- 15	LIP 1.4 Rep 3.2	060101	10 plum	GAK 1	frozen	
- 16	DW 1.4 Rep 1.1	060101	10 flem	GAK 1	frozen	
- 17	DW 1.4 Rep 2.1	060101	10 flem	GAK 1	frozen	
- 18	DW 1.4 Rep 3.1	060101	10 flem	GAK 1	frozen	
- 19	DW 1.4 Rep 1.2	060101	10 plum	GAK 1	frozen	
- 20	DW 1.4 Rep 2.2	060101	10 plum	GAK 1	frozen	
- 21	DW 1.4 Rep 3.2	060101	10 plum	GAK 1	frozen	

Appendix I (cont.).

- 22	TIS 1.4 R1 con	060201	10 flem	GAK 1	frozen
- 23	TIS 1.4 R2 con	060201	10 flem	GAK 1	frozen
- 24	TIS 1.4 R3 con	060201	10 flem	GAK 1	frozen
- 25	TIS 1.4 R1 low	060201	10 flem	GAK 1	frozen
- 26	TIS 1.4 R2 low	060201	10 flem	GAK 1	frozen
- 27	TIS 1.4 R3 low	060201	10 flem	GAK 1	frozen
- 28	TIS 1.4 R1 high	060201	10 flem	GAK 1	frozen
- 29	TIS 1.4 R2 high	060201	10 flem	GAK 1	frozen
- 30	TIS 1.4 R3 high	060201	10 flem	GAK 1	frozen
- 31	TIS 1.4 R1 con Plum	060201	10 plum	GAK 1	frozen
- 32	TIS 1.4 R2 con Plum	060201	10 plum	GAK 1	frozen
- 33	TIS 1.4 R3 con Plum	060201	10 plum	GAK 1	frozen
- 34	TIS 1.4 R1 low Plum	060201	10 plum	GAK 1	frozen
- 35	TIS 1.4 R2 low Plum	060201	10 plum	GAK 1	frozen
- 36	TIS 1.4 R3 low Plum	060201	10 plum	GAK 1	frozen
- 37	TIS 1.4 R1 high Plum	060201	10 plum	GAK 1	frozen
- 38	TIS 1.4 R2 high Plum	060201	10 plum	GAK 1	frozen
- 39	TIS 1.4 R3 high Plum	060201	10 plum	GAK 1	frozen
- 40	W1.4 control	060201	water T24	Lab	900 mL
- 41	W1.4 low	060201	water T24	Lab	900 mL
- 42	W1.4 high	060201	water T24	Lab	900 mL
- 43	W3.2 control	060401	water T0	Lab	900 mL
- 44	W3.2 low	060401	water T0	Lab	900 mL
- 45	W3.2 med	060401	water T0	Lab	900 mL
- 46	W3.2 high	060401	water T0	Lab	900 mL
- 47	W3.2 control	060501	water T24	Lab	900 mL
- 48	W3.2 low	060501	water T24	Lab	900 mL
- 49	W3.2 med	060501	water T24	Lab	900 mL
- 50	W3.2 high	060501	water T24	Lab	900 mL

Appendix 2. PAC concentrations in exposure water (Chapter 2). Exp. = experiment; T0, T24 = start and end time of oil exposure

Controls	Exp.1 T0	Exp. 1 T24	Exp. 2 T0	Exp. 2 T24, Exp. 3 T0	Exp. 3 T24	Exp. 4 T0	Exp. 4 T24
id =	1106407	1106410	1106434	1106443	1200905	1203206	1203240
qcbatch =	R10041	R10041	R10041	R10041	R10051	R10041	R10041
vol (l)=	0.9	0.9	0.9	0.9	0.9	0.9	0.9
matrix =	WATER	WATER	WATER	WATER	WATER	WATER	WATER
Analyte conc.(ng/l):							
naphthalene	176.92	99.73	68.25	69.09	61.88	80.70	54.37
N1	220.20	84.70	54.24	49.59	35.97	54.71	37.48
C-2 naphthalenes	182.88	62.25	46.63	41.99	31.79	32.65	26.23
C-3 naphthalenes	302.07	119.04	86.09	77.03	44.07	46.18	32.73
C-4 naphthalenes	187.81	81.43	50.73	45.30	26.98	20.31	14.32
biphenyl	39.50	16.07	12.74	12.25	9.13	13.87	9.51
acenaphthylene	0.00	0.00	0.00	0.00	0.00	0.00	0.00
acenaphthene	185.97	66.85	43.22	38.62	28.98	51.57	35.91
fluorene	154.02	61.94	43.86	38.59	24.95	33.03	24.96
C-1 fluorenes	86.06	62.12	65.24	47.62	34.37	17.38	16.19
C-2 fluorenes	109.57	66.50	45.59	34.41	20.69	18.31	16.80
C-3 fluorenes	79.45	17.12	18.20	11.15	0.00	0.00	0.00
dibenzothiophene	28.77	13.65	10.65	9.19	6.42	9.09	7.58
C-1 dibenzothioph.	30.45	4.24	4.57	4.15	5.80	8.91	7.53
C-2 dibenzothioph.	17.34	7.74	6.53	5.55	4.61	7.04	5.00
C-3 dibenzothioph.	37.48	20.71	6.33	5.77	6.84	6.23	2.93
phenanthrene	339.52	143.50	103.34	88.62	58.16	68.76	53.60
C-1 phenanth./anthr.	210.23	88.38	62.75	55.18	35.51	32.90	26.30
C-2 phenanth./anthr.	138.17	66.02	43.54	37.21	24.81	18.67	14.59
C-3 phenanth./anthr.	56.73	23.15	13.49	11.49	8.07	7.77	5.46
C-4 phenanth./anthr.	20.37	11.66	0.00	6.34	0.00	0.00	2.04
anthracene	0.00	0.00	0.00	0.00	0.00	0.00	0.00
fluoranthene	47.70	20.23	15.93	11.78	7.62	10.05	8.09
pyrene	28.63	10.36	7.97	5.97	3.74	4.54	3.66
C-1 fluoranth./pyrenes	13.64	11.17	5.91	4.83	3.58	3.42	2.58
benz-a-anthracene	0.00	0.00	0.00	0.00	0.96	0.00	0.00
chrysene	6.60	0.00	0.00	0.00	0.00	0.00	0.00
C-1 chrysenes	5.56	0.00	0.00	0.00	0.00	0.00	0.00
C-2 chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C-3 chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C-4 chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00
benzo-b-fluoranthene	0.00	0.00	0.00	0.00	0.00	0.00	0.00
benzo-k-fluoranthene	0.00	0.00	0.00	0.00	0.00	0.00	0.00
benzo-e-pyrene	0.00	0.00	0.00	0.00	0.00	0.00	0.00
benzo-a-pyrene	0.00	0.00	0.00	0.00	0.00	0.00	0.00
perylene	0.00	0.00	0.00	0.00	0.00	0.00	0.00
indeno-123-cd-pyrene	0.00	0.00	0.00	0.00	0.00	0.00	0.00
dibenzo-a,h-anthr.	0.00	0.00	0.00	0.00	0.00	0.00	0.00
benzo-g,h,i-perylene	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total PAC	2841.46	1207.34	849.14	744.46	504.82	566.82	422.35

Appendix 2 (cont.)

Low dose	Exp.1 T0	Exp. 1 T24	Exp. 2 T0	Exp. 2 T24, Exp. 3 T0	Exp. 3 T24	Exp. 4 T0	Exp. 4 T24
id =	1106405	1106408	1106435	1106444	1203207	1203241	1200906
qcbatch =	R10041	R10041	R10041	R10041	R10041	R10041	R10051
vol (l)=	0.9	0.9	0.9	0.9	0.9	0.9	0.9
matrix =	WATER	WATER	WATER	WATER	WATER	WATER	WATER
catno =	RMB_081	RMB_081	RMB_081	RMB_081	RMB_081	RMB_081	RMB_081
Analyte conc.(ng/l):							
naphthalene	222.98	80.90	76.61	67.13	86.08	66.06	66.97
N1	294.33	76.70	63.72	43.69	59.58	38.24	38.91
C-2 naphthalenes	339.20	129.51	126.97	113.15	95.64	72.04	92.98
C-3 naphthalenes	997.38	800.48	851.02	839.05	640.96	522.71	831.99
C-4 naphthalenes	788.31	617.61	572.16	570.03	470.21	384.47	593.53
biphenyl	44.47	14.48	13.62	11.00	15.18	9.88	9.47
acenaphthylene	2.05	0.00	0.00	0.00	0.00	0.00	0.00
acenaphthene	196.55	50.94	48.52	27.83	65.60	39.61	25.66
fluorene	242.36	92.47	102.32	76.12	89.04	58.64	61.21
C-1 fluorenes	452.89	331.00	395.34	321.66	294.68	216.93	315.19
C-2 fluorenes	607.47	447.31	413.48	399.67	366.95	286.53	418.06
C-3 fluorenes	283.36	197.48	176.86	173.92	159.46	127.30	180.75
dibenzothiophene	357.87	328.90	294.73	298.38	250.97	220.69	271.90
C-1 dibenzothiophenes	553.88	544.76	451.05	483.34	408.08	373.95	498.40
C-2 dibenzothiophenes	312.88	296.71	220.92	258.27	225.37	206.09	285.66
C-3 dibenzothiophenes	115.02	81.02	64.72	70.11	61.64	55.78	85.45
phenanthrene	1006.50	666.12	595.05	553.42	490.79	406.86	506.94
C-1 phenanthr./anthr.	1458.00	1271.15	1043.92	1091.79	937.79	841.54	1143.08
C-2 phenanthr./anthr.	798.49	637.95	510.96	537.69	465.79	409.70	591.67
C-3 phenanthr./anthr.	253.65	161.28	120.50	121.97	109.70	97.42	137.70
C-4 phenanthr./anthr.	31.79	22.27	15.80	12.86	10.87	8.94	16.27
anthracene	0.00	6.93	0.00	6.64	0.00	4.67	0.00
fluoranthene	73.72	24.43	20.97	13.47	15.00	11.06	11.60
pyrene	57.90	24.94	20.59	16.48	15.80	11.99	16.24
C-1 fluoranth./pyrenes	91.93	47.70	35.30	32.59	30.08	23.24	34.47
benz-a-anthracene	11.48	1.89	1.50	0.00	0.00	0.00	1.90
chrysene	32.04	18.13	14.64	14.02	11.87	11.07	16.77
C-1 chrysenes	44.88	16.82	12.87	10.47	10.17	6.77	11.79
C-2 chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C-3 chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C-4 chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00
benzo-b-fluoranthene	9.55	0.00	0.00	0.00	0.00	0.00	0.00
benzo-k-fluoranthene	9.84	0.00	0.00	0.00	0.00	0.00	0.00
benzo-e-pyrene	0.00	0.00	0.00	0.00	0.00	0.00	0.00
benzo-a-pyrene	29.65	0.00	0.00	0.00	0.00	0.00	0.00
perylene	0.00	0.00	0.00	0.00	0.00	0.00	0.00
indeno-123-cd-pyrene	0.00	0.00	0.00	0.00	0.00	0.00	0.00
dibenzo-a,h-anthr.	22.74	0.00	0.00	0.00	0.00	0.00	0.00
benzo-g,h,i-perylene	19.15	0.00	0.00	0.00	0.00	0.00	0.00
Total PAC	9762.28	6989.89	6264.12	6164.73	5387.31	4512.17	6264.56

Appendix 2 (cont.).

High dose	Exp.1 T0	Exp. 1 T24	Exp. 2 T0	Exp. 2 T24, Exp. 3 T0	Exp. 3 T24	Exp. 4 T0	Exp. 4 T24
id =	1106406	1106409	1106436	1106445	1200907	1203208	1203242
qcbatch =	R10041	R10041	R10041	R10041	R10051	R10041	R10041
vol (l)=	0.9	0.9	0.9	0.9	0.9	0.9	0.9
matrix =	WATER	WATER	WATER	WATER	WATER	WATER	WATER
catno =	RMB_081	RMB_081	RMB_081	RMB_081	RMB_081	RMB_081	RMB_081
Analyte conc. (ng/l):							
naphthalene	157.17	132.15	62.23	63.27	92.24	61.81	71.65
N1	335.91	314.56	62.53	64.64	56.38	39.33	40.10
C-2 naphthalenes	1474.68	1510.85	494.50	526.69	547.75	102.76	97.08
C-3 naphthalenes	2698.08	2705.04	1967.47	2038.51	2210.12	995.75	916.15
C-4 naphthalenes	1199.09	1196.05	900.84	926.45	994.40	623.27	498.98
biphenyl	67.28	65.14	14.97	15.44	14.84	9.90	10.76
acenaphthylene	0.00	0.00	0.00	0.00	0.00	0.00	0.00
acenaphthene	107.39	92.12	33.31	27.79	26.96	37.37	48.32
fluorene	428.41	414.94	194.42	192.81	181.64	114.80	104.92
C-1 fluorenes	850.37	918.33	604.54	598.88	634.26	472.09	419.32
C-2 fluorenes	870.67	860.30	567.17	585.80	618.70	509.24	446.28
C-3 fluorenes	327.19	366.08	229.51	248.66	253.01	217.54	181.00
dibenzothiophene	1019.17	998.69	659.81	691.79	725.88	600.82	539.53
C-1 dibenzothioph.	807.95	791.18	647.15	686.47	765.65	651.98	578.37
C-2 dibenzothioph.	372.34	331.08	287.14	310.14	347.46	306.79	268.54
C-3 dibenzothioph.	69.93	52.93	62.06	66.59	81.25	66.94	60.92
phenanthrene	1617.24	1627.08	1021.13	1067.27	1115.74	968.24	857.16
C-1 phenanthr./anthr.	1792.79	1802.08	1369.70	1478.16	1627.60	1430.89	1267.85
C-2 phenanthr./anthr.	772.55	738.06	553.14	618.24	673.50	583.40	516.42
C-3 phenanthr./anthr.	208.73	194.64	128.17	147.94	159.60	144.36	124.27
C-4 phenanthr./anthr.	27.57	26.66	14.11	17.26	15.77	14.71	12.08
anthracene	10.58	0.00	8.80	9.63	8.79	0.00	0.00
fluoranthene	38.41	37.88	12.26	12.09	9.94	12.21	12.04
pyrene	35.25	34.15	16.41	17.56	17.68	15.52	14.29
C-1 fluoranth./pyrenes	65.15	62.00	33.08	36.30	35.69	35.20	32.11
benz-a-anthr.	5.37	5.53	0.00	0.00	0.00	0.00	0.00
chrysene	23.37	21.38	13.70	15.25	17.15	13.46	12.34
C-1 chrysenes	30.23	26.27	10.65	13.54	12.50	8.15	7.49
C-2 chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C-3 chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C-4 chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00
benzo-b-fluoranth.	8.59	6.25	0.00	0.00	0.00	0.00	0.00
benzo-k-fluoranthene	0.00	0.00	0.00	0.00	0.00	0.00	0.00
benzo-e-pyrene	0.00	0.00	0.00	0.00	0.00	0.00	0.00
benzo-a-pyrene	5.86	9.25	0.00	0.00	0.00	0.00	0.00
perylene	0.00	0.00	0.00	0.00	0.00	0.00	0.00
indeno-123-cd-pyrene	0.00	0.00	0.00	0.00	0.00	0.00	0.00
dibenzo-a,h-anthr.	0.00	0.00	0.00	0.00	0.00	0.00	0.00
benzo-g,h,i-perylene	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total PAC	15427.33	15340.65	9968.81	10477.17	11244.49	8036.52	7137.96

Appendix 3. PAC concentrations in copepod tissue (Chapter 2). String = set of samples in analytical process; see Appendix 2 for unabbreviated PAC analytes.

Experiment 1

Aromatic Data	High			Low			Control		
Strng 101501SD									
sample ID	1106414	1106415	1106416	1106411	1106412	1106413	1106417	1106418	1106419
wet weight (g)	0 0232	0 0224	0 0247	0 0305	0 0262	0 0236	0 0036	0 0184	0 0302
matrix	TISSUE	TISSUE	TISSUE	TISSUE	TISSUE	TISSUE	TISSUE	TISSUE	TISSUE
Surrogate Recovenes (%)									
NAPHTHALENE d-8	80 47	86 20	80 89	89 73	66 84	88 04	98 43	93 62	81 96
ACENAPHTHENE d-10	81 81	87 87	83 11	87 22	67 92	89 98	99 97	93 28	81 78
PHENANTHRENE d-10	81 93	89 58	84 74	92 52	69 88	90 30	101 26	94 81	81 89
CHRYSENE d-12	77 29	85 06	80 98	82 51	66 22	84 63	93 59	83 73	77 03
BENZO-a-PYRENE d-12	84 24	94 81	96 85	66 65	78 89	92 49	89 82	81 89	94 41
PERYLENE d-12	86 21	91 68	96 74	79 21	82 10	94 97	91 19	86 85	86 65
Analyte concentrations (ng g)							data error		
N0	0 00	0 00	0 00	0 00	0 00	0 00		0 00	0 00
N1	177 83	164 89	163 78	0 00	0 00	0 00		0 00	0 00
N2	1008 12	869 20	1201 76	107 69	151 63	181 87		127 74	88 59
N3	2458 39	2091 73	2866 91	606 14	832 35	1070 59		169 46	78 50
N4	600 60	750 36	865 29	278 26	364 05	575 55		0 00	0 00
bip	0 00	0 00	0 00	0 00	0 00	0 00		0 00	0 00
ace	0 00	0 00	0 00	0 00	0 00	0 00		0 00	0 00
acn	200 06	0 00	136 93	0 00	134 26	153 14		195 38	106 20
F0	294 62	0 00	300 80	0 00	0 00	0 00		0 00	0 00
F1	712 83	665 77	892 72	283 84	379 22	455 42		0 00	0 00
F2	639 53	585 47	794 40	344 05	484 24	620 10		0 00	0 00
F3	0 00	0 00	0 00	0 00	0 00	0 00		0 00	0 00
D0	745 86	681 69	949 95	242 86	310 57	354 71		0 00	0 00
D1	845 78	784 26	999 34	463 00	677 28	854 65		0 00	0 00
D2	502 46	457 53	551 25	276 33	491 10	591 67		0 00	0 00
D3	180 41	0 00	207 20	0 00	185 98	229 41		0 00	0 00
P0	1659 37	1327 96	1666 81	666 44	790 08	921 27		255 87	211 20
P1	1644 67	1502 80	1995 89	1764 78	1317 09	1708 52		0 00	0 00
P2	1019 84	914 29	1107 73	782 53	1031 12	1269 64		0 00	0 00
P3	0 00	0 00	0 00	0 00	0 00	0 00		0 00	0 00
P4	0 00	0 00	0 00	0 00	0 00	0 00		0 00	0 00
ant	0 00	0 00	0 00	0 00	0 00	0 00		0 00	0 00
fla	0 00	0 00	0 00	0 00	0 00	0 00		0 00	0 00
pyr	0 00	0 00	0 00	0 00	0 00	0 00		0 00	0 00
C1fp	0 00	0 00	0 00	0 00	0 00	0 00		0 00	0 00
baa	0 00	0 00	0 00	0 00	0 00	0 00		0 00	0 00
C0	0 00	0 00	0 00	0 00	0 00	0 00		0 00	0 00
C1	0 00	0 00	0 00	0 00	0 00	0 00		0 00	0 00
C2	0 00	0 00	0 00	0 00	0 00	0 00		0 00	0 00
C3	0 00	0 00	0 00	0 00	0 00	0 00		0 00	0 00
C4	0 00	0 00	0 00	0 00	0 00	0 00		0 00	0 00
bbf	0 00	0 00	0 00	0 00	0 00	0 00		0 00	0 00
bkf	0 00	0 00	0 00	0 00	0 00	0 00		0 00	0 00
bep	0 00	0 00	0 00	0 00	0 00	0 00		0 00	0 00
bap	0 00	0 00	0 00	0 00	0 00	0 00		0 00	0 00
per	0 00	0 00	0 00	0 00	0 00	0 00		0 00	0 00
icp	0 00	0 00	0 00	0 00	0 00	0 00		0.00	0 00
daa	0 00	0 00	0 00	0 00	0 00	0 00		0 00	0 00
bgp	0 00	0 00	0 00	0 00	0 00	0 00		0 00	0 00
Total PAC	12690 36	10795 95	14700 78	5815.93	7148 96	8986 53		748 46	484 48
Mean	12729			7317				616	
Stand. dev.	1953			1592				187	

Appendix 3 (cont.)

Experiment 2

Aromatic Data String 101501SD	High			Low			Control		
sample ID	1200902	1200903	1200904	1106449	1106450	1200901	1106446	1106447	1106448
wetwt (g)=	0 0336	0 0263	0 0296	0 0278	0 0302	0 0327	0 0296	0 0302	0 0313
matrix =	TISSUE	TISSUE	TISSUE	TISSUE	TISSUE	TISSUE	TISSUE	TISSUE	TISSUE
Surrogate Recoveries (%)									
NAPHTHALENE d-8	84 80	82 76	78 20	86 86	90 51	114 66	84 22	93 31	82 06
ACENAPHTHENE d-10	85 68	85 12	82 99	89 55	92 59	112 18	88 77	95 64	87 87
PHENANTHRENE d-10	88 62	89 03	87 17	91 37	92 67	115 75	93 21	98 51	92 47
CHRYSENE d-12	95 08	79 60	95 31	93 45	82 41	111 54	89 22	94 02	92 60
BENZO-a-PYRENE d-12	103 17	81 81	96 20	98 91	92 76	126 85	101 58	99 14	95 42
PERYLENE d-12	97 66	86 44	98 39	96 21	90 40	126 38	98 86	97 78	92 71
Analyte concentrations (ng/g)									
N0	0 00	244 58	0 00	0 00	0 00	0 00	0 00	0 00	0 00
N1	0 00	0 00	0 00	143 91	0 00	0 00	0 00	0 00	0 00
N2	759 10	929 05	908 50	266 07	228 66	180 03	104 07	95 06	102 35
N3	3301 03	3894 05	3810 93	1680 67	1371 13	1410 18	96 89	96 57	85 45
N4	939 87	1332 13	1410 58	813 03	509 88	544 90	0 00	0 00	0 00
bip	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
ace	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
acn	0 00	0 00	0 00	0 00	0 00	0 00	123 99	0 00	0 00
F0	247 70	292 45	278 15	0 00	0 00	0 00	0 00	0 00	0 00
F1	1135 60	1249 94	1295 54	624 31	525 19	587 82	0 00	0 00	0 00
F2	1101 93	1313 16	1344 24	884 75	777 03	842 39	0 00	0 00	0 00
F3	219 52	0 00	328 29	227 82	0 00	231 58	0 00	0 00	0 00
D0	1029 59	1272 99	1250 06	511 49	457 79	466 21	0 00	0 00	0 00
D1	1330 36	1536 33	1568 06	1086 51	928 17	967 02	0 00	0 00	0 00
D2	789 38	874 63	902 95	744 21	587 63	677 90	0 00	0 00	0 00
D3	273 45	308 08	302 57	249 66	254 92	266 53	0 00	0 00	0 00
P0	1779 14	2160 28	2109 57	1068 83	910 62	909 41	0 00	0 00	0 00
P1	2599 70	2876 16	3040 78	2247 81	1891 22	2009 78	0 00	0 00	0 00
P2	1589 16	1806 63	1819 56	1487 06	1265 59	1442 00	0 00	0 00	0 00
P3	339 73	325 43	412 00	0 00	0 00	293 63	0 00	0 00	0 00
P4	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
ant	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
fla	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
pyr	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
C1fp	221 19	0 00	0 00	0 00	0 00	0 00	0 00	0 00	258 19
baa	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
C0	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	140 81
C1	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
C2	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
C3	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
C4	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
bbf	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
bkf	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
bep	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
bap	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
per	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
icp	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
daa	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
bgp	0 00	0 00	0 00	0 00	640 76	0 00	0 00	0 00	0 00
Total PAC	17656 45	20415 90	20781 78	12036 13	10348 61	10829 38	324 95	191 62	586 81
Mean	19618			11071			368		
Stand. dev.	1709			869			201		

Appendix 3 (cont.)

Experiment 3

Aromatic Data	High			Low			Control		
Stnng 101501SD									
sample ID	1200914	1200915	1200916	1200911	1200912	1200913	1200908	1200909	1200910
wetwt (g)=	0 0451	0 0447	0 0526	0 049	0 0487	0 0596	0 0339	0 0455	0 0465
matnx =	TISSUE	TISSUE	TISSUE	TISSUE	TISSUE	TISSUE	TISSUE	TISSUE	TISSUE
Surrogate Recovenes (%)									
NAPHTHALENE d-8	88 75	85 17	95 28	83 56	84 08	91 46	84 11	90 02	86 27
ACENAPHTHENE d-10	90 56	87 75	93 68	87 40	87 43	90 96	85 68	89 36	87 56
PHENANTHRENE d-10	91 38	90 37	94 64	91 02	90 66	90 53	88 69	91 17	89 07
CHRYSENE d-12	100 14	97 07	101 78	98 40	84 04	98 74	95 61	98 77	96 06
BENZO-a-PYRENE d-12	103 24	101 86	107 86	104 99	96 34	102 15	101 44	108 92	101 42
PERYLENE d-12	102 34	100 38	104 92	105 92	96 21	99 43	99 36	101 49	99 17
Analyte concentrations (ng.g)									
N0	150 43	162 25	118 40	132.34	128 51	111 42	219 62	138 36	133 62
N1	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
N2	582 56	683 52	524 32	98 27	130 23	123 77	106 03	66 31	92 25
N3	2289 30	2599 47	1963 32	822 92	897 96	706 00	153 71	95 30	114 48
N4	800 53	839 76	651 92	292 57	375 67	292 33	0 00	0 00	0 00
bip	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
ace	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
acn	57 97	80 84	0 00	57 83	0 00	0 00	82 66	87 32	83 72
F0	192 34	215 58	172 85	0 00	0 00	0 00	0 00	0 00	0 00
F1	816 15	929 37	691 31	367 52	355 73	279 62	0 00	0 00	0 00
F2	760 89	875 61	529 98	301 58	570 34	419 34	0 00	145 66	0 00
F3	139 27	189 07	159 86	0 00	130 75	0 00	0 00	0 00	0 00
D0	753 34	849 74	668 23	247 94	268 85	213 30	0 00	0 00	0 00
D1	922 97	1038 50	786 43	531 31	578 98	451 28	0 00	0 00	0 00
D2	519 22	571 41	436 81	372 01	406 49	315 23	0 00	0 00	0 00
D3	175 72	188 28	134 42	157 64	166 88	109 53	0 00	0 00	0 00
P0	1303 98	1456 05	1129 47	552 91	579 67	459 10	136 18	87 85	92 40
P1	1744 38	1960 03	1509 56	1011 19	1139 40	871 48	0 00	0 00	0 00
P2	996 22	1101 16	817 67	720 56	832 82	596 76	0 00	0 00	0 00
P3	242 44	235 44	192 79	180 10	0 00	144 53	0 00	0 00	0 00
P4	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
ant	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
fla	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
pyr	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
C1fp	317 94	285 15	0 00	0 00	0 00	0 00	0 00	0 00	0 00
baa	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
C0	0 00	0 00	0 00	0 00	0 00	0 00	0 00	101 61	0 00
C1	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
C2	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
C3	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
C4	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
bbf	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
bkf	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
bep	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
bap	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
per	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
icp	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
daa	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
bgp	0 00	0.00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
Total PAC	12765 65	14261 22	10487 33	5846 70	6562.28	5093 69	698 19	712 41	516 48
Mean	12505			5834			642		
Stand. dev.	1900			734			109		

Appendix 3 (cont.)

Experiment 4

Aromatic Data	High			Low			Control		
Stnng 101501SD									
sample ID	1203228	1203229	1203230	1203225	1203226	1203227	1203222	1203223	1203224
wetwt (g)=	0 03	0 0242	0 0268	0 03	0 03	0 03	0 0239	0 023	0 0209
matnx =	TISSUE	TISSUE	TISSUE	TISSUE	TISSUE	TISSUE	TISSUE	TISSUE	TISSUE
Surrogate Recoveries (%)									
NAPHTHALENE d-8	93 05	87 89	84 64	91 68	74 35	89 91	86 05	85 00	20 80
ACENAPHTHENE d-10	89 45	89 12	86 82	90 66	77 00	86 45	89 02	86 53	21 42
PHENANTHRENE d-10	89 77	90 79	88 07	99 16	71 70	85 05	90 55	87 50	19 09
CHRYSENE d-12	86 10	81 11	85 14	89 11	69 84	82 97	91 27	83 25	18 51
BENZO-a-PYRENE d-12	95 93	80 79	94 23	94 93	79 43	93 10	97 11	95 19	22 40
PERYLENE d-12	91 68	86 43	95 22	99 52	76 52	92 27	93 50	98 10	22 14
Analyte concentrations (ng/g)									
N0	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
N1	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	195 65
N2	173 22	220 47	236 68	150 12	161 81	108 54	94 03	123 42	250 01
N3	1590 89	1808 48	2137 29	1204 81	1102 59	821 40	241 00	100 16	204 53
N4	595 13	710 01	1011 94	533 01	562 44	458 88	0 00	0 00	0 00
bip	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
ace	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
acn	128 11	154 45	136 50	124 08	118 44	122 07	0 00	0 00	0 00
F0	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
F1	696 04	761 56	893 02	504 79	398 96	277 67	0 00	0 00	0 00
F2	805 05	844 76	1016 80	692 37	581 94	449 13	0 00	0 00	0 00
F3	0 00	0 00	224 12	0 00	234 96	0 00	0 00	0 00	0 00
D0	695 07	753 12	929 43	367 30	337 77	228 85	0 00	0 00	0 00
D1	1062 67	1185 07	1378 10	817 72	822 44	588 40	0 00	0 00	0 00
D2	642 93	738 99	817 75	599 06	523 43	447 42	0 00	0 00	0 00
D3	224 09	269 04	288 48	222 41	0 00	172 97	0 00	0 00	0 00
P0	1311 19	1497 29	1729 45	787 45	752 42	529 76	0 00	0 00	0 00
P1	2198 01	2310 58	2744 64	1664 13	1860 24	1182 18	0 00	0 00	0 00
P2	1264 04	1524 09	1635 67	1206 10	1244 44	893 62	0 00	0 00	0 00
P3	0 00	0 00	340 59	0 00	0 00	183 49	0 00	0 00	0 00
P4	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
ant	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
fla	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
pyr	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	449 10
C1fp	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
baa	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
C0	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
C1	0 00	0 00	281 69	0 00	0 00	0 00	0 00	0 00	0 00
C2	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
C3	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
C4	0 00	0 00	207 61	0 00	0 00	0 00	0 00	0 00	0 00
bbf	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
bkf	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
bep	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
bap	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
per	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	1119 49
icp	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
daa	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
bgp	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
Total PAC	11386 45	12777 91	16009 75	8873 35	8701 85	6464 39	335 03	223 58	2218 77
Mean	13391			8013			926		
Stand. dev.	2372			1344			1121		

Appendix 4. Lipid content, wet and dry weights, lipid composition of *Neocalanus*. (Chapter 2): Lipid content table shows sample wet weights and lipid weights after extraction and drying. Lipid % dry weight is calculated with the dry weights from wet and dry weights table (below). See Appendix 1 for sample ID and abbreviations.

Lipid content							gravimetric method						
ID	vial full	vial empty		Mean	St Dev	lipid wgt	Mean	St dev	lipid% dry wt	sampling date	location	sample size	species
11064 02	3 21278	3 19312	0 01966			0 00120						9	
03	3 2411	3 22043	0 02067			0 00134						10	
04	3 22302	3 20238	0 02064	0 0203	0 0006	0 00110	0 00121	0 00012	16 94*	041601	PWS	10	N flem
11064 27	3 15983	3 13203	0 02780			0 00394						10	
28	3 17662	3 15068	0 02594			0 00260						10	
29	3 17729	3 13484	0 04245	0 0321	0 0090	0 00376	0 00343	0 00073	87 59**	050101	GAK1	10	N flem
11064 31	3 12846	3 0936	0 03486			0 00470						9	
32	3 15317	3 11344	0 03973			0 00626						10	
33	3 17945	3 13292	0 04653	0 0404	0 0059	0 00622	0 00573	0 00089	79 94	050201	PWS	10	N flem
12009 17	3 13907	3 12131	0 01776			0 00126						10	
18	3 11241	3 09678	0 01563			0 00090						10	
19	3 13641	3 11812	0 01829	0 0172	0 0014	0 00096	0 00104	0 00019	84 10***	051401	Cape Cleare	10	N flem
12009 22	3 15951	3 13394	0 02557			0 00118						10	
23	3 18837	3 1665	0 02187			0 00092						10	
24	3 19811	3 15083	0 04728	0 0316	0 0137	0 00104	0 00105	0 00013	84 64***	051401	Cape Cleare	10	N plum
12032 10	3 16029	3 13629	0 02400			0 00168						10	
12	3 18762	3 16588	0 02174			0 00110						10	
13	3 19357	3 16599	0 02758	0 0244	0 0029	0 00112	0 00130	0 00033	41 01	060101	GAK1	10	N flem
12032 14	3 17521	3 14824	0 02697			0 00124						10	
11	3 17941	3 15103	0 02838			0 00134						10	
15	3 17506	3 14066	0 03440	0 0299	0 0039	0 00120	0 00126	0 00007	40 21	060101	GAK1	10	N plum

* Mean dry weight used from PWS early May sample.
**Mean dry weight used from GOA sample
***Same dry weight from mixed species sample

Appendix 4 (cont.)

Wet and dry weights

ID		sample wet wt	mean	St Dev	dry wt (24hrs)	dry wt (27hrs)	mean	St dev	% moist	mean % moist	St dev	date	location species*
11064	37	0 01163			0 00621	0 00617			47				
	38	0 01771			0 00746	0 00744			58				
	39	0 00921	0 01285	0 00438	0 00807	0 00788	0 00716	0 00089	14	40	22 64	050201	PWS
11064	40	0 01131			0 00406	0 00388			66				
	41	0 01133			0 00336	0 00307			73				
	42	0 00901	0 01055	0 00133	0 00512	0 00481	0 00392	0 00087	47	62	13 58	050301	GOA
12009	21	0 00322			0 00221	0 00195			39				
	45	0 00290			0 00110	0 00096			67				
	46	0 00702	0 00438	0 00229	0 00099	0 00080	0 00124	0 00062	89	65	24 64	051401	Cape Cleare
12032	16	0 00845			0 00404	0 00397			53				
	17	0 01246			0 00312	0 00284			77				
	18	0 01039	0 01043	0 00201	0 00310	0 00270	0 00317	0 00070	74	68	13 14	060101	GAK1
12032	19	0 00694			0 00386	0 00373			46				
	20	0 00858			0 00272	0 00254			70				
	21	0 01287	0 00946	0 00306	0 00314	0 00313	0 00313	0 00060	76	64	15 69	060101	GAK1 N plum

* *Neocalanus flemingeri* unless otherwise indicated. N. plum. = *N. plumchrus*

Lipid composition

Listed as mg analyte in 1 g tissue, then as mg analyte in 1 g lipid. SIN = sample ID as listed in chain of custody sheets (Appendix 1). QCBatch is a Laboratory internal identification; note that % lipid are wet weight based. CE/WE = Cholesterol esters/wax esters; TAG = triacylglycerides, CHO = cholesterol; MON = monoacylglyceride; FFA = free fatty acids; PE = phosphatidylethanolamine; PC = phosphatidylcholine.

Compound Sample Concentration (mg/g tissue)

SIN	1203211	1203214	1203215	1106431	1106432	1106433	1203210	1106402	1106403	1106404
QCBatch	D032702	D032702	D032702	D032702	D032702	D032702	D032702	D032702	D032702	D032702
Replicate #	1	1	1	1	1	1	1	2	1	1
Sample Wt (g)	0 03	0 03	0 03	0 03	0 04	0 05	0 02	0 02	0 02	0 02
% Lipid	4 72	4 60	3 49	13 48	15 76	13 37	7 00	6 10	6 48	5 33
Dry Wt (g)	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000
CE / WE	28 223	29 972	22 975	79 215	98 376	78 872	47 340	42 617	43 769	39 850
TAG	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000
Cholest	4 829	5 078	3 968	4 080	3 879	3 249	0 000	0 000	0 000	6 597
Monolein	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000
FFA	3 784	3 888	3 064	4 799	6 083	4 495	0 000	5 276	0 000	4 795
PE	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000
PC	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000
mg analytes in 1 g tissue =	**36.837**	**38.938**	**30.007**	**88.094**	**108.339**	**86.617**	**47.340**	**47.893**	**43.769**	**51.242**

Appendix 4 (cont.)

SIN	1200917	1200918	1200919	1106427	1106428	1203213	1106429	1200923	1203212	1200922	1200924
QCBatch	D032702	D032702	D032702	D032702	D032702	D032702	D032702	D032702	D032702	D032702	D032702
Replicate #	1	1	1	1	1	1	1	1	1	1	1
Sample Wt (g)	0 02	0 02	0 02	0 03	0 03	0 03	0 04	0 02	0 02	0 03	0 05
% Lipid	7 09	5 76	5 25	14 17	10 02	4 06	8 86	4 21	5 06	4 61	2 20
Dry Wt (g)	0 000	0 000	0 000	0 000	1 000	0 000	0 000	0 000	0 000	0 000	0 000
CE/WE	43 735	32 185	28 619	88 852	63 126	24 841	63 435	23 636	36 376	29 078	11 360
TAG	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000
Cholest	0 000	8 706	0 000	5 119	5 342	4 953	3 422	6 228	0 000	5 336	2 883
Monolein	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000
FFA	5 746	6 502	5 662	5 897	5 288	3 687	4 401	4 926	0 000	4 377	2 398
PE	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000
PC	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000
mg analytes in 1 g tissue	**49.481**	**47.392**	**34.281**	**99.868**	**73.755**	**33.480**	**71.258**	**34.790**	**36.376**	**38.791**	**16.642**

mg component / g lipid

SIN	1203211	1203214	1203215	1106431	1106432	1106433	1203210	1106402	1106403	1106404
CE / WE	597 95	651 57	658 31	587 65	624 22	589 92	676 29	698 63	675 45	747 65
TAG	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
Cholest	102 32	110 39	113 71	30 27	24 61	24 30	0 00	0 00	0 00	123 77
Monolein	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
FFA	80 17	84 53	87 78	35 60	38 60	33 62	0 00	86 49	0 00	89 96
PE	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
PC	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
mg analytes in 1 g lipid =	**780.44**	**846.49**	**859.80**	**653.52**	**687.43**	**647.84**	**676.29**	**785.12**	**675.45**	**961.38**

SIN	1200917	1200918	1200919	1106427	1106428	1203213	1106429	1200923	1203212	1200922	1200924
CE/WE	616 86	558 77	545 13	627 04	630 00	611 85	715 97	561 43	718 89	630 77	516 37
TAG	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
Cholest	0 00	151 14	0 00	36 13	53 31	121 99	38 62	147 92	0 00	115 74	131 06
Monolein	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
FFA	81 05	112 88	107 85	41 61	52 77	90 80	49 68	117 01	0 00	94 95	109 02
PE	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
PC	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
mg analytes in 1 g lipid	**697.90**	**822.78**	**652.98**	**704.78**	**736.08**	**824.64**	**804.27**	**826.37**	**718.89**	**841.46**	**756.45**

Appendix 5. Report on creosote contamination in Resurrection Bay water.

Chemical analysis of seawater from Resurrection Bay in Seward reveals creosote contamination

Switgard Duesterloh, Juneau Center School of Fisheries and Ocean Sciences, University of Alaska Fairbanks, 11120 Glacier Highway, Juneau, Alaska 99801, USA

A series of 15 seawater samples in four time intervals of 3-4 samples each was collected from Resurrection Bay in Seward between April 18 and June 5, 2001 and analyzed for polyaromatic compound (PAC) content. All samples were collected as seawater "blanks" in a series of experiments conducted to determine biological effects of various concentrations of oil derived PAC on plankton. The sampling periods were: April 18-21, May 02-04, May 15-19 and June 01-05.

Seawater was directed from the laboratory supply line into an overhead tank of approximately 80 Liter capacity. It was then pumped through a column of PAC cleaned glass beads at a flow rate of 5 ml/min into a 2 Liter Erlenmeyer flask. Ninehundred ml were taken from the Erlenmeyer flask and immediately extracted twice with 50 - 60 ml dichloromethane and frozen until further analysis at the Auke Bay Laboratory (NMFS/NOAA). Dichloromethane extracts of the PAC were reduced in volume and exchanged with hexane over a steam bath. PAC were measured by gas chromatography/mass spectrometry (GC/MS) in the selected ion monitoring mode (SIM). PAC analytes included dibenzothiophenes and polyaromatic hydrocarbons containing 2-5 rings, including the alkylated homologues listed in Figure 1. A method blank, spiked method blank, and two reference samples were analyzed with each batch of 12 samples to verify method accuracy, precision, and absence of laboratory introduced artifacts and interferences. Detection limits were determined experimentally (1) for PAC and generally

Appendix 5 (cont.).

were 5-20 ng PAC/L seawater. Concentrations below the detection limit were treated as 0.

All samples had a distinctive PAC signature which was identified as typical for creosote contamination (Fig. 2). Total PAC concentrations ranged from 422 to 2841 ng/Liter and averaged 913 ng/Liter. PAC concentrations in this magnitude have been reported to cause genetic damage to fish embryos (2). The location of the water intake pipe is in the vicinity of a former large railroad dock and warehouse structure which was destroyed by a tidal wave during the large earthquake in 1964 and collapsed into the Bay. Some of the old railroad ties can still be found on the property of the present marine research laboratory of the University of Alaska.

Literature cited

1) Glaser, J.A., Forest, D.L., McKee, G.D., Quave, S.A. & Budde, W.L. *Environ. Sci. Technol.* **1981**, 15, 1426-1435.

2) Rice, S.D., Thomas, R.E., Carls, M.G., Heintz, R.A., Wertheimer, A.C., Murphy, M.L., Short, J.W., Moles, A. *Reviews in Fisheries Science* **2001**, 9(3), 165-211.

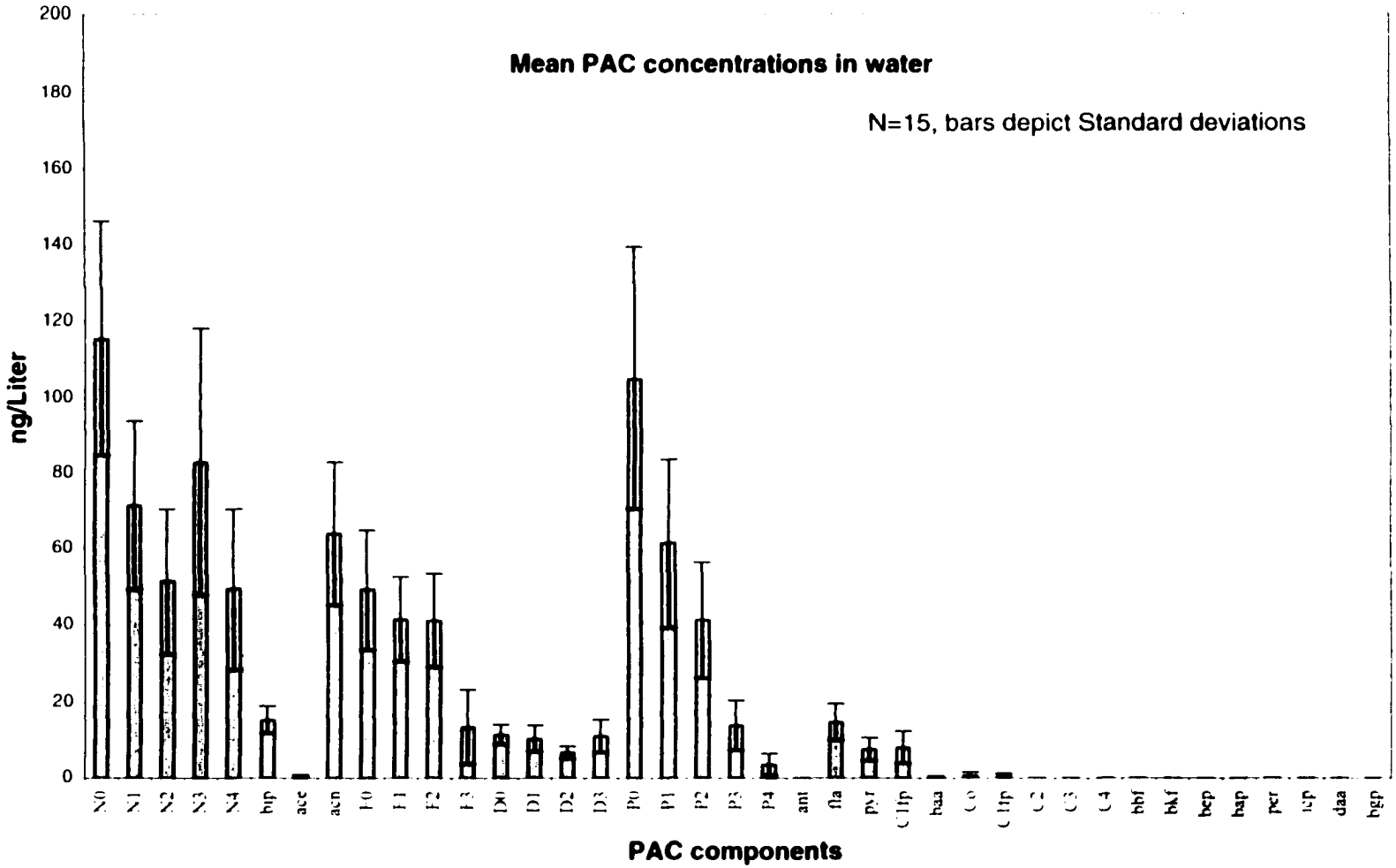
Appendix 5 (cont.).

Figures:

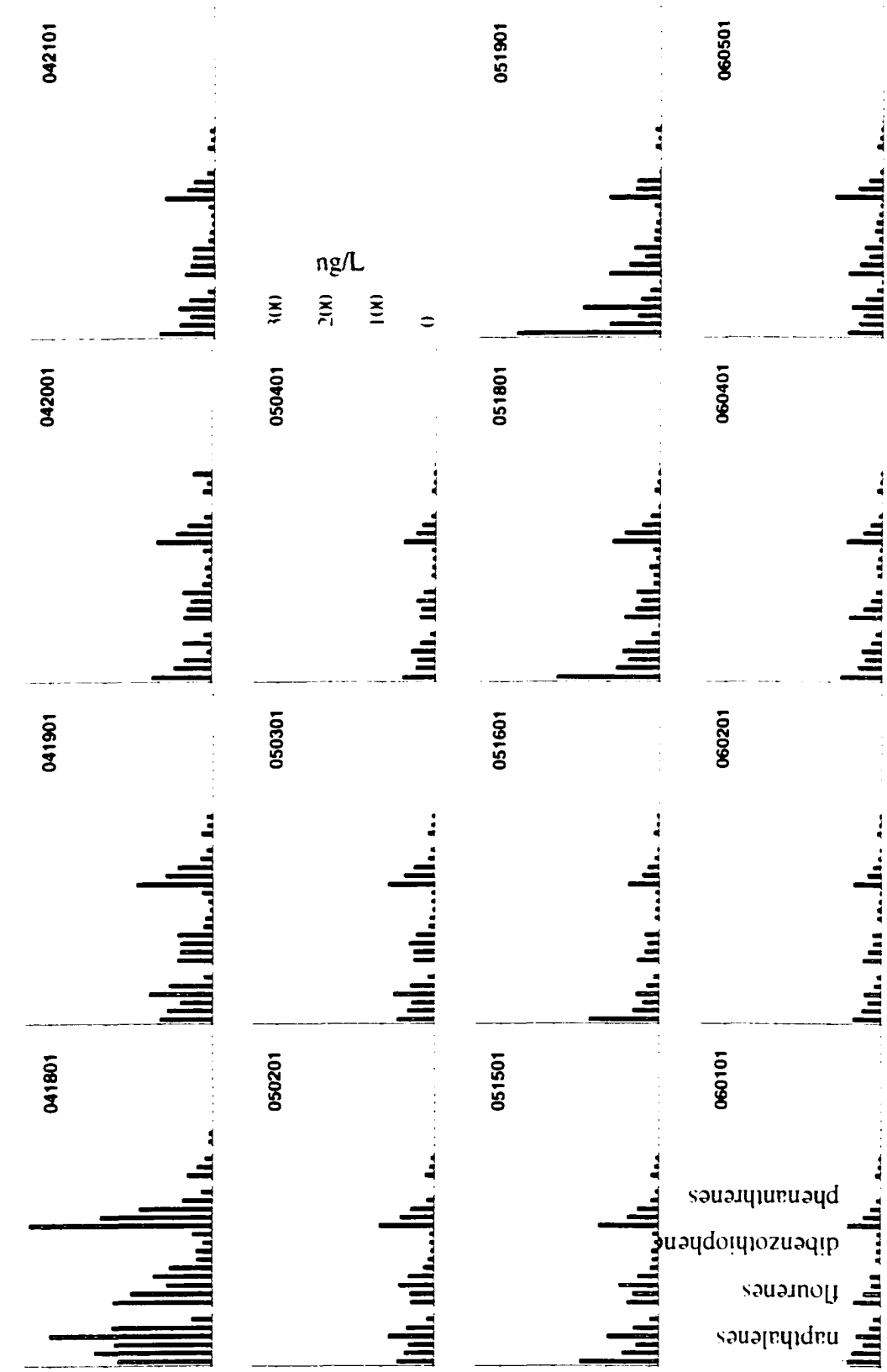
Fig. 1: Mean polyaromatic compound (PAC) concentrations in 15 water samples from Resurrection Bay, Seward, AK collected between April 16, 2001 and June 5, 2001. Bars depict standard deviations. N, F, D, P and C refer to naphthalene, flourene, dibenzothiophene, phenanthrene and chrysene, respectively, and the numbers following these letters indicate the number of alkyl-substituent carbon atoms. Other PAC are abbreviated as follows: bip = biphenyl, ace = acenaphthylene, acn = acenapthene, ant = anthracene, fla = flouranthene, pyr = pyrene, C1fp = C1 flouranthenes/pyrenes, baa = benz-a-anthracene, bbf = benzo-b-flouranthene, bkf = benzo-k-flouranthene, bep = benzo-e-pyrene, bap = benzo-a-pyrene, per = perylene, icp = indeno-1,2,3-c,d-pyrene, daa = dibenzo-a,h-anthracene, bgp = benzo-g,h,i-perylene.

Fig. 2: PAC concentrations in individual water samples. The scale depicted in the upper right hand graph refers equally to all graphs. The sequence of PAC compounds on the X-axes is equivalent to that shown in Fig. 1.

Appendix 5 (cont.)



Appendix 5 (cont.).



Appendix 6. PAC concentrations and proportions of individual analytes in exposure water. (Chapter 3)

Analytes (ng/l)	Exp 1 tissue residue T0	pro portion	tissue residue T24	pro portion	control T0	pro portion	control T24	pro portion	Oil no UV T0	pro portion	Oil no UV T24	pro portion	Oil+UV T0	pro portion	Oil + UV T24	pro portion
naphtalene	21 49	0 01	27 91	0 01	19 20	0 10	17 40	0 12	34 37	0 02	24 87	0 01	25 69	0 02	20 82	0 01
2-methylnaphthalene	10 93	0 01	10 67	0 00	11 29	0 06	9 70	0 07	20 38	0 01	12 46	0 00	13 87	0 01	11 71	0 00
1-methylnaphthalene	5 36	0 00	5 30	0 00	5 49	0 03	4 93	0 04	8 97	0 00	5 88	0 00	6 50	0 01	6 27	0 00
C-2 naphthalenes	20 47	0 01	23 01	0 01	16 17	0 09	12 36	0 09	30 80	0 02	20 33	0 01	19 79	0 02	24 98	0 01
C-3 naphthalenes	121 06	0 07	179 81	0 06	32 54	0 17	19 85	0 14	150 94	0 08	155 94	0 06	106 66	0 09	178 04	0 07
C-4 naphthalenes	84 04	0 05	153 87	0 05	10 98	0 06	5 47	0 04	81 45	0 04	115 82	0 05	60 61	0 05	151 91	0 06
biphenyl	3 33	0 00	3 38	0 00	4 23	0 02	3 45	0 02	4 76	0 00	4 03	0 00	4 21	0 00	3 74	0 00
acenaphthylene	0 45	0 00	0 14	0 00	0 39	0 00	0 45	0 00	0 46	0 00	0 27	0 00	0 59	0 00	0 57	0 00
acenaphthene	3 99	0 00	3 48	0 00	5 04	0 03	4 40	0 03	0 00	0 00	3 61	0 00	0 00	0 00	6 90	0 00
fluorene	10 93	0 01	14 44	0 00	7 61	0 04	6 62	0 05	14 02	0 01	13 86	0 01	12 82	0 01	17 10	0 01
C-1 fluorenes	56 44	0 03	98 63	0 03	4 87	0 03	4 51	0 03	63 22	0 03	76 01	0 03	43 04	0 03	85 14	0 03
C-2 fluorenes	123 05	0 07	204 60	0 07	12 46	0 07	10 54	0 08	136 04	0 07	170 31	0 07	87 28	0 07	193 31	0 07
C-3 fluorenes	83 33	0 05	126 00	0 04	4 29	0 02	3 62	0 03	42 60	0 02	113 24	0 04	23 10	0 02	119 03	0 04
dibenzothiophene	60 53	0 04	104 04	0 04	1 60	0 01	1 18	0 01	54 07	0 03	78 02	0 03	35 48	0 03	83 58	0 03
C-1 dibenzothiophenes	157 29	0 09	268 24	0 09	2 74	0 01	1 98	0 01	178 24	0 09	222 91	0 09	110 68	0 09	231 51	0 09
C-2 dibenzothiophenes	121 10	0 07	201 82	0 07	2 40	0 01	1 65	0 01	126 02	0 07	181 35	0 07	77 94	0 06	184 43	0 07
C-3 dibenzothiophenes	33 76	0 02	62 19	0 02	0 71	0 00	0 27	0 00	29 21	0 02	52 45	0 02	18 99	0 02	53 89	0 02
phenanthrene	118 49	0 07	192 91	0 07	16 21	0 09	12 29	0 09	123 09	0 06	160 79	0 06	79 29	0 06	177 30	0 07
C-1 phenanthrenes/an	358 06	0 21	643 40	0 22	8 99	0 05	6 02	0 04	433 23	0 23	542 35	0 21	277 62	0 22	563 24	0 21
C-2 phenanthrenes/an	221 21	0 13	408 46	0 14	6 49	0 03	3 39	0 02	251 30	0 13	388 95	0 15	144 54	0 12	393 36	0 15
C-3 phenanthrenes/an	64 67	0 04	119 44	0 04	2 89	0 02	2 60	0 02	76 96	0 04	110 12	0 04	51 42	0 04	109 85	0 04
C-4 phenanthrenes/an	0 00	0 00	15 99	0 01	0 00	0 00	0 00	0 00	10 06	0 01	18 31	0 01	6 20	0 01	13 89	0 01
anthracene	0 69	0 00	1 25	0 00	0 32	0 00	0 31	0 00	0 00	0 00	0 99	0 00	0 00	0 00	1 95	0 00
fluoranthene	4 89	0 00	4 65	0 00	3 53	0 02	2 59	0 02	0 63	0 00	6 48	0 00	5 24	0 00	12 36	0 00
pyrene	6 40	0 00	11 46	0 00	1 54	0 01	1 17	0 01	0 00	0 00	1 04	0 00	5 69	0 00	14 49	0 01
C-1 flouranthenes/pyre	11 92	0 01	24 39	0 01	0 00	0 00	0 33	0 00	13 30	0 01	21 80	0 01	5 39	0 00	22 25	0 01
benz-a-anthracene	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
chrysene	10 03	0 01	16 26	0 01	0 00	0 00	0 00	0 00	8 02	0 00	16 10	0 01	6 00	0 00	15 44	0 01
C-1 chrysenes	0 00	0 00	4 81	0 00	0 00	0 00	0 00	0 00	2 59	0 00	4 85	0 00	1 12	0 00	0 00	0 00
C-2 chrysenes	11 47	0 01	6 40	0 00	5 80	0 03	2 10	0 02	12 94	0 01	3 64	0 00	9 86	0 01	0 00	0 00
C-3 chrysenes	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
C-4 chrysenes	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
benzo-b-flouranthene	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 37	0 00	0 00	0 00
benzo-k-flouranthene	0 31	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
benzo-e-pyrene	0 00	0 00	0 00	0 00	0 00	0 00	0 35	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
benzo-a-pyrene	0 00	0 00	0 34	0 00	0 00	0 00	0 07	0 00	0 38	0 00	0 00	0 00	0 12	0 00	0 18	0 00
perylene	0 00	0 00	0 00	0 00	0 00	0 00	0 06	0 00	0 15	0 00	0 00	0 00	0 00	0 00	0 07	0 00
indeno-123 cd-pyrene	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
dibenzo-a,h-anthracene	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
benzo-g,h,i-pyrylene	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
Total PAH	1726		2937		188		140		1908		2527		1240		2697	

Appendix 6 (cont.).

analytes (ng/L)	Exp 2 tissue residue T0	pro-portion	tissue residue T24	pro-portion	control T0	pro-portion	control T24	pro-portion	Oil, no UV T0	pro-portion	Oil, no UV T24	pro-portion	Oil + UV T0	pro-portion	Oil + UV T24	pro-portion
naphtalene	18 67	0 01	23 45	0 01	16 55	0 12	15 33	0 17	19 33	0 01	16 17	0 01	18 37	0 01	16 48	0 01
2-methylnaphthalene	9 89	0 00	10 86	0 00	8 42	0 06	8 02	0 09	10 20	0 00	8 84	0 00	8 67	0 00	7 92	0 00
1-methylnaphthalene	4 85	0 00	5 51	0 00	4 32	0 03	4 20	0 05	4 55	0 00	3 39	0 00	4 26	0 00	3 97	0 00
C-2 naphthalenes	20 98	0 01	20 38	0 01	13 15	0 09	8 55	0 09	20 06	0 01	8 38	0 00	15 44	0 01	15 48	0 01
C-3 naphthalenes	160 63	0 07	135 77	0 06	31 17	0 22	15 52	0 17	127 61	0 06	98 15	0 05	115 31	0 06	102 04	0 05
C-4 naphthalenes	122 63	0 05	104 46	0 04	6 27	0 04	2 88	0 03	90 23	0 04	112 94	0 06	91 12	0 05	101 38	0 05
biphenyl	2 91	0 00	3 21	0 00	2 85	0 02	2 83	0 03	3 27	0 00	3 02	0 00	2 63	0 00	2 77	0 00
acenaphthylene	0 20	0 00	0 23	0 00	0 47	0 00	0 35	0 00	0 31	0 00	0 34	0 00	0 31	0 00	0 30	0 00
acenaphthene	2 81	0 00	5 81	0 00	3 75	0 03	0 00	0 00	2 95	0 00	3 19	0 00	3 63	0 00	3 57	0 00
fluorene	11 53	0 00	12 42	0 01	5 53	0 04	4 30	0 05	9 45	0 00	6 88	0 00	10 65	0 01	8 02	0 00
C-1 fluorenes	85 85	0 04	108 36	0 04	3 59	0 03	2 36	0 03	63 74	0 03	52 61	0 03	63 07	0 03	55 43	0 03
C-2 fluorenes	172 91	0 07	172 61	0 07	10 99	0 08	7 01	0 08	143 63	0 07	146 80	0 07	131 67	0 07	137 63	0 07
C-3 fluorenes	100 99	0 04	111 86	0 05	0 00	0 00	0 90	0 01	93 64	0 04	102 30	0 05	80 97	0 04	87 09	0 04
dibenzothiophene	88 86	0 04	67 64	0 03	0 92	0 01	0 79	0 01	61 55	0 03	40 40	0 02	62 88	0 03	50 71	0 03
C-1 dibenzothiophenes	228 78	0 09	224 31	0 09	1 72	0 01	1 05	0 01	185 67	0 09	180 12	0 09	166 93	0 09	181 61	0 09
C-2 dibenzothiophenes	163 34	0 07	168 01	0 07	1 44	0 01	0 60	0 01	150 60	0 07	173 50	0 09	127 66	0 07	149 06	0 08
C-3 dibenzothiophenes	49 97	0 02	49 19	0 02	0 00	0 00	0 00	0 00	50 34	0 02	57 37	0 03	39 08	0 02	44 74	0 02
phenanthrene	163 29	0 07	134 28	0 06	9 50	0 07	6 99	0 08	111 65	0 05	69 07	0 03	121 22	0 06	100 86	0 05
C-1 phenanthrenes/an	556 56	0 23	544 89	0 22	4 64	0 03	3 37	0 04	440 59	0 21	405 34	0 20	402 95	0 21	430 78	0 22
C-2 phenanthrenes/an	291 23	0 12	350 63	0 14	3 51	0 02	1 29	0 01	318 26	0 15	342 03	0 17	278 06	0 15	309 61	0 16
C-3 phenanthrenes/an	100 16	0 04	97 79	0 04	0 78	0 01	1 02	0 01	99 29	0 05	100 38	0 05	78 38	0 04	77 27	0 04
C-4 phenanthrenes/an	13 00	0 01	15 34	0 01	0 00	0 00	0 00	0 00	17 07	0 01	11 99	0 01	10 92	0 01	11 75	0 01
anthracene	0 99	0 00	1 01	0 00	0 20	0 00	0 12	0 00	4 66	0 00	1 18	0 00	1 03	0 00	0 89	0 00
fluoranthene	3 98	0 00	4 11	0 00	1 97	0 01	1 37	0 01	3 71	0 00	3 63	0 00	5 54	0 00	5 01	0 00
pyrene	10 05	0 00	9 92	0 00	0 88	0 01	0 61	0 01	8 78	0 00	9 83	0 00	8 69	0 00	9 29	0 00
C-1 flouranthenes/pyre	20 91	0 01	20 84	0 01	0 48	0 00	0 00	0 00	20 03	0 01	20 81	0 01	16 33	0 01	17 84	0 01
benz-a-anthracene	0 00	0 00	0 00	0 00	0 60	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
chrysene	14 31	0 01	13 66	0 01	0 22	0 00	0 24	0 00	12 82	0 01	15 06	0 01	11 28	0 01	12 82	0 01
C-1 chrysenes	1 72	0 00	6 31	0 00	0 00	0 00	0 00	0 00	3 25	0 00	9 25	0 00	1 88	0 00	6 06	0 00
C-2 chrysenes	10 99	0 00	3 02	0 00	6 75	0 05	2 26	0 02	11 68	0 01	2 94	0 00	10 23	0 01	3 37	0 00
C-3 chrysenes	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
C-4 chrysenes	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
benzo-b-flouranthene	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 58	0 00	0 00	0 00	0 27	0 00
benzo-k-flouranthene	0 00	0 00	0 25	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
benzo-e-pyrene	0 00	0 00	0 00	0 00	0 03	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
benzo-a-pyrene	0 12	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 12	0 00	0 36	0 00	0 07	0 00	0 06	0 00
perylene	0 00	0 00	0 00	0 00	0 07	0 00	0 00	0 00	0 06	0 00	0 00	0 00	0 09	0 00	0 00	0 00
indeno-123-cd-pyrene	0 00	0 00	0	0 00	0	0 00	0	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
dibenzo-a,h-anthracene	0 00	0 00	0	0 00	0	0 00	0	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
benzo-g,h,i-pyrylene	0 00	0 00	0	0 00	0	0 00	0	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00	0 00
Total PAH	2433		2426		141		92		2089		2007		1889		1954	

Appendix 7. PAC concentrations in *Calanus marshallae* and *Metridia okhotensis* tissue. (Chapter 3); C. marsh. = *Calanus marshallae*; Metridia = *Metridia okhotensis*; MDL = method detection limits on a 80% significance level; sample ID refers to chain of custody identification (Appendix 1)

		Experiment	1	1	1	1
		Species (N)	C. marsh. (16)	C. marsh. (16)	C. marsh. (15)	C. marsh. (15)
		Exposure	+ Oil	- Oil, + UV	+ Oil, - UV	+ Oil, + UV
		Treatment	before light			
		sample ID	1106309	1106310	1106311	1106312
		wetweight (g)	0.0055	0.0058	0.0188	0.0062
		matrix	TISSUE	TISSUE	TISSUE	TISSUE
Surrogate Recoveries(%):						
NAPHTHALENE d-8			44.17	35.04	49.81	35.71
ACENAPHTHENE d-10			66.84	59.10	71.46	56.15
PHENANTHRENE d-10			75.20	83.72	80.49	81.77
CHRYSENE d-12			79.66	91.49	86.68	89.92
BENZO-a-PYRENE d-12			62.15	69.36	65.14	65.35
PERYLENE d-12			70.35	85.63	79.01	83.03
MASS (ng)	MDL Amount					
naphthalene	1.72		2.86	4.61	3.93	4.47
2-methylnaphth.	1.09		1.16	2.24	1.68	1.30
1-methylnaphth.	2.83		0.00	0.00	0.00	0.00
C-2 naphthalenes	0.57		0.71	0.00	0.00	0.00
C-3 naphthalenes	0.68		10.39	1.29	3.97	5.90
C-4 naphthalenes	0.68		12.51	0.00	0.00	0.00
biphenyl	2.39		0.00	0.00	0.00	0.00
acenaphthylene	1.92		0.00	0.00	0.00	0.00
acenaphthene	0.78		0.00	0.00	0.00	0.00
fluorene	1.77		0.00	0.00	0.00	0.00
C-1 fluorenes	1.77		5.67	0.00	2.03	2.91
C-2 fluorenes	1.77		14.04	2.66	5.03	7.27
C-3 fluorenes	1.77		5.19	0.00	0.00	0.00
dibenzothiophene	1.14		4.71	0.00	1.38	2.37
C-1 dibenzothiophenes	1.14		18.20	0.00	6.46	10.26
C-2 dibenzothiophenes	1.14		16.80	2.40	6.30	8.95
C-3 dibenzothiophenes	1.14		5.90	2.06	1.86	2.73
phenanthrene	1.11		13.04	1.67	4.92	7.41
C-1 phenanthr./anthr.	2.53		57.04	6.74	18.55	31.90
C-2 phenanthr./anthr.	2.53		43.71	9.35	13.65	22.10
C-3 phenanthr./anthr.	2.53		13.79	6.63	4.18	6.14
C-4 phenanthr./anthr.	2.53		2.72	0.00	0.00	0.00
anthracene	0.89		0.00	0.00	0.00	0.00
fluoranthene	2.08		0.00	0.00	0.00	0.00
pyrene	2.14		0.00	0.00	0.00	0.00
C-1 fluoranthenes/pyrenes	2.14		3.13	0.00	0.00	0.00
benz-a-anthracene	0.74		0.00	0.00	0.00	0.00
chrysene	1.18		3.51	1.86	0.00	1.57
C-1 chrysenes	1.18		1.81	1.44	0.00	0.00
C-2 chrysenes	1.18		2.26	1.74	0.00	0.00
C-3 chrysenes	1.18		0.00	0.00	0.00	0.00
C-4 chrysenes	1.18		0.00	0.00	0.00	0.00
benzo-b-fluoranthene	3.38		0.00	0.00	0.00	0.00
benzo-k-fluoranthene	1.67		0.00	0.00	0.00	0.00
benzo-e-pyrene	1.99		0.00	0.00	0.00	0.00
benzo-a-pyrene	1.6		0.00	0.00	0.00	0.00

Appendix 7 (cont.)

perylene	2.25		0 00	0 01	0 00	0.00
indeno-123-cd-pyrene	1.06		0 00	0.00	0.00	0.00
dibenzo-a,h-anthracene	1 32		0.00	0.00	0.00	0 00
benzo-g,h,i-perylene	3.69		0.00	0.00	0.00	0.00
TOTAL			239	45	74	115
Total per individual			14 95	2.79	4 93	7 69

	Experim	2	2	2	2	2
	Spec (N)	C mars (20)	Metridia (40)	C marsh (20)	C marsh (21)	C marsh (21)
	Oil Exp	24 h. + Oil	24 h. + Oil	24 h. - Oil. + UV	24 h. + Oil. - UV	24 h. + Oil. + UV
	Treatm	before light	before light			
	sampleID	1106321	1106322	1106323	1106324	1106325
	wet weight (g)	0 0278	0 0112	0 0096	0 021	0 0119
	matrix	TISSUE	TISSUE	TISSUE	TISSUE	TISSUE
Surrogate Recoveries(%)						
NAPHTHALENE d-8		25 09	35 38	34 80	24 99	37 91
ACENAPHTHENE d-10		50 97	63 35	59 11	45 99	65 49
PHENANTHRENE d-10		82 31	84 87	82 91	76 82	85 41
CHRYSENE d-12		87 94	89 90	88 79	80 74	90 05
BENZO-a-PYRENE d-12		67 78	72 66	64 84	59 48	67 26
PERYLENE d-12		81 31	76 91	73 43	67 23	72 44
MASS (ng)	MDL Amount					
naphthalene	1 72	10 08	6 43	12 76	18 36	6 82
2-methylnaphth	1 09	4 98	2 54	5 28	6 68	2 77
1-methylnaphth	2 83	2 10	0 00	2 49	3 24	0 00
C-2 naphthalenes	0 57	1 42	0 00	2 59	1 30	0 00
C-3 naphthalenes	0 68	12 63	11 19	1 63	8 58	8 58
C-4 naphthalenes	0 68	0 00	0 00	0 00	11 65	8 22
biphenyl	2 39	0 00	0 00	0 00	0 00	0 00
acenaphthylene	1 92	0 00	0 00	0 00	0 00	0 00
acenaphthene	0 78	0 00	0 00	0 00	0 00	0 00
fluorene	1 77	0 00	0 00	0 00	0 00	0 00
C-1 fluorenes	1 77	8 23	7 05	0 00	6 53	2 99
C-2 fluorenes	1 77	20 63	18 44	0 00	21 60	8 70
C-3 fluorenes	1 77	5 59	4 42	0 00	7 21	2 71
dibenzothiophene	1 14	5 09	5 02	0 00	2 93	3 20
C-1 dibenzothiophenes	1 14	23 79	23 33	0 00	20 62	17 93
C-2 dibenzothiophenes	1 14	21 48	19 00	0 00	23 35	16 90
C-3 dibenzothiophenes	1 14	6 77	5 10	0 00	7 62	5 07
phenanthrene	1 11	12 64	13 57	0 00	7 80	10 35
C-1 phenanthr /anthr	2 53	58 37	63 73	0 00	49 83	50 89
C-2 phenanthr /anthr	2 53	40 62	41 56	0 00	44 15	37 50
C-3 phenanthr /anthr	2 53	11 57	10 50	0 00	13 60	10 08
C-4 phenanthr /anthr	2 53	0 00	0 00	0 00	0 00	0 00
anthracene	0 89	0 00	0 00	0 00	0 00	0 00
fluoranthene	2 08	0 00	0 00	0 00	0 00	0 00
pyrene	2 14	0 00	0 00	0 00	0 00	0 00
C-1 fluoranthenes/pyrenes	2 14	2 23	2 26	0 00	2 40	0 00
benz-a-anthracene	0 74	0 00	0 00	0 00	0 00	0 00
chrysene	1 18	2 67	2 51	2 08	3 29	2 76
C-1 chrysenes	1 18	0 00	0 00	0 00	0 00	0 00
C-2 chrysenes	1 18	0 00	0 00	0 00	0 00	0 00
C-3 chrysenes	1 18	0 00	0 00	0 00	0 00	0 00
C-4 chrysenes	1 18	0 00	0 00	0 00	0 00	0 00
benzo-b-fluoranthene	3 38	0 00	0 00	0 00	0 00	0 00
benzo-k-fluoranthene	1 67	0 00	0 00	0 00	0 00	0 00
benzo-e-pyrene	1 99	0 00	0 00	0 00	0 00	0 00

Appendix 7 (cont.)

benzo-a-pyrene	1 6	0 00	0 00	0 00	0 00	0 00
perylene	2 25	0 00	0 00	0 00	0 00	0 00
indeno-123-cd-pyrene	1 06	0 00	0 00	0 00	0 00	0 00
dibenzo-a.h-anthracene	1 32	0 00	0 00	0 00	0 00	0 00
benzo-g.h.i-perylene	3 69	0 00	0 00	0 00	0 00	0 00
TOTAL		251	237	27	261	195
Total per individual		12 55	5 92	1 34	12 42	9 31

Appendix 8. PAC concentrations in exposure water (Chapter 4). T0, T24 = start and end time of oil exposure; SD = Standard deviation

Experiment 1

	T0 control	T24 control	T0 low	T24 low	T0 medium	T24 medium	T0 high	T24 high
sample ID	1200925	1200929	1200926	1200930	1200927	1200931	1200928	1200932
volume (l)=	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
matrix =	WATER	WATER	WATER	WATER	WATER	WATER	WATER	WATER
Surrogate Recoveries (%)								
NAPHTHALENE d-8	80.64	83.80	78.18	68.83	79.04	85.31	70.64	71.03
ACENAPHTHENE d-10	88.24	89.75	88.38	78.26	88.97	89.73	79.44	87.32
PHENANTHRENE d-10	88.82	85.27	91.46	80.77	91.19	88.98	85.52	90.03
CHRYSENE d-12	83.70	77.48	85.20	70.38	85.76	85.30	80.99	91.35
BENZO-a-PYRENE d-12	106.94	96.54	108.53	75.71	115.97	111.65	94.82	111.50
PERYLENE d-12	92.43	89.52	95.59	76.70	95.85	96.67	96.08	101.81
Analyte conc. (ng/l)								
naphthalene	151.85	133.31	145.81	161.08	131.02	130.23	171.59	150.26
C-1 naphthalenes	69.53	50.95	69.29	68.40	60.82	54.49	79.63	70.24
C-2 naphthalenes	43.28	31.90	56.48	38.65	100.67	75.92	361.18	265.79
C-3 naphthalenes	97.83	44.13	225.17	213.11	507.90	483.74	1712.95	1172.77
C-4 naphthalenes	48.57	24.21	160.66	135.95	390.28	361.29	743.46	559.98
biphenyl	16.68	11.79	13.92	13.17	13.52	12.19	17.49	14.22
acenaphthylene	0.00	0.00	0.00	1.89	0.00	0.00	0.00	2.50
acenaphthene	61.55	41.88	55.27	56.86	50.26	46.11	54.37	50.71
fluorene	50.63	27.30	50.99	43.66	71.56	57.03	240.73	182.61
C-1 fluorenes	76.33	22.98	105.60	79.42	252.54	200.96	627.01	465.38
C-2 fluorenes	39.81	27.10	123.05	109.55	307.56	280.90	691.84	524.22
C-3 fluorenes	15.50	0.00	54.61	44.36	131.88	119.56	299.74	226.86
dibenzothiophene	12.52	7.68	66.66	57.75	194.45	179.37	706.36	549.35
C-1 dibenzothiophenes	13.51	6.93	126.46	109.75	332.02	328.10	617.39	484.92
C-2 dibenzothiophenes	6.35	5.69	75.83	67.22	177.63	186.73	309.20	242.25
C-3 dibenzothiophenes	11.07	7.29	27.14	23.17	51.12	51.33	65.58	52.99
phenanthrene	114.54	59.35	191.05	163.24	396.66	350.51	1093.53	859.66
C-1 phenanthr./anthr.	59.79	30.93	311.38	259.82	772.51	751.36	1382.65	1091.42
C-2 phenanthr./anthr.	40.76	21.07	170.86	140.02	383.79	379.80	594.30	475.85
C-3 phenanthr./anthr.	15.30	7.07	43.89	35.99	92.76	89.20	160.21	128.55
C-4 phenanthr./anthr.	5.61	3.03	8.30	7.01	10.43	8.97	20.59	20.93
anthracene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
fluoranthene	15.50	10.34	13.64	15.64	12.68	11.20	14.56	12.91
pyrene	7.36	3.85	8.99	7.84	14.13	12.83	17.38	14.26
C-1 fluoranth./pyrenes	5.68	3.62	11.62	9.79	25.20	23.03	41.75	35.50
benz-a-anthr.	0.00	0.00	0.00	0.00	0.79	0.00	2.62	0.00
chrysene	0.00	0.00	4.69	4.47	9.87	9.07	20.09	11.78
C-1 chrysenes	0.00	0.00	3.11	0.00	8.17	6.52	15.43	12.60
C-2 chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C-3 chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C-4 chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
benzo-b-fluoranthene	0.00	0.00	0.00	0.00	0.00	0.00	7.06	0.00
benzo-k-fluoranthene	0.00	0.00	0.00	0.00	0.00	0.00	5.47	0.00
benzo-e-pyrene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
benzo-a-pyrene	0.00	0.00	0.00	0.00	3.00	0.00	6.43	0.00
perylene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
indeno-123-cd-pyrene	0.00	0.00	0.00	0.00	0.00	0.00	15.62	0.00
dibenzo-a,h-anthracene	0.00	0.00	0.00	0.00	0.00	0.00	22.73	0.00
benzo-g,h,i-perylene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total PAC	1013.28	601.28	2124.46	1867.83	4503.24	4210.43	10118.95	7678.50
	mean	SD	mean	SD	mean	SD	mean	SD
	807	291	1996	181	4357	207	8899	1726

Appendix 8 (cont.).

Experiment 2

	T0 control	T24 control	T0 low	T24 low	T0 medium	T24 medium	T0 high	T24 high
id =	1203243	1203247	1203244	1203248	1203245	1203249	1203246	1203250
qcbatch =	R05021	R10051	R10051	R10051	R10051	R10051	R10051	R10051
vol (l)=	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
matrix =	WATER	WATER	WATER	WATER	WATER	WATER	WATER	WATER
catno =	RMB_081	RMB_081	RMB_081	RMB_081	RMB_081	RMB_081	RMB_081	RMB_081
Surrogate Recoveries (%)								
NAPHTHALENE d-8	84.66	91.06	74.58	75.26	79.16	93.00	82.38	72.00
ACENAPHTHENE d-10	90.54	96.45	84.76	80.75	84.76	93.44	88.32	75.90
PHENANTHRENE d-10	84.59	91.11	84.46	77.07	88.85	93.17	87.49	75.18
CHRYSENE d-12	74.04	84.84	74.86	72.79	82.47	90.55	91.73	77.66
BENZO-a-PYRENE d-12	76.40	107.92	93.67	97.84	107.89	113.56	109.22	95.42
PERYLENE d-12	73.57	95.64	84.00	83.97	96.11	94.00	113.93	92.26
Analyte conc (ng/l)								
naphthalene	79.44	67.05	78.43	60.83	85.34	70.10	94.82	97.30
C-1 naphthalenes	47.54	47.09	47.87	36.29	46.95	34.29	64.19	57.23
C-2 naphthalenes	31.58	37.12	69.46	46.81	99.35	75.67	313.24	295.28
C-3 naphthalenes	38.49	59.40	583.90	429.13	1035.85	764.43	1170.01	1072.14
C-4 naphthalenes	19.37	32.51	455.41	365.49	674.17	516.92	566.06	518.16
biphenyl	12.54	12.32	12.39	10.31	11.62	13.33	19.96	18.44
acenaphthylene	0.00	2.03	1.71	1.79	1.76	0.00	0.00	0.00
acenaphthene	62.44	55.40	52.61	50.24	64.10	57.56	48.38	55.71
fluorene	35.78	43.96	65.54	49.96	124.16	91.67	235.98	208.62
C-1 fluorenes	21.97	25.12	265.45	179.67	546.49	381.68	518.08	444.70
C-2 fluorenes	12.62	34.38	370.33	286.27	578.64	412.78	809.20	602.23
C-3 fluorenes	0.00	6.58	178.53	112.81	265.33	186.55	329.49	250.32
dibenzothiophene	9.66	14.65	243.07	147.69	602.97	424.18	719.54	617.28
C-1 dibenzothiophenes	11.37	12.02	475.33	357.65	710.81	517.35	694.13	554.68
C-2 dibenzothiophenes	7.28	6.29	277.47	221.49	334.08	244.48	358.86	281.20
C-3 dibenzothiophenes	4.33	4.00	80.03	69.92	82.29	57.79	118.10	52.04
phenanthrene	67.75	90.91	465.78	306.38	997.23	708.20	1068.47	931.00
C-1 phenanthr./anthr.	36.75	46.23	1091.97	806.23	1580.72	1136.51	1444.18	1228.90
C-2 phenanthr./anthr.	22.81	25.86	567.76	444.67	671.82	487.27	674.90	546.30
C-3 phenanthr./anthr.	6.33	4.45	134.22	106.13	156.30	108.14	203.72	145.16
C-4 phenanthr./anthr.	0.00	0.00	14.37	10.42	17.67	10.53	45.74	22.15
anthracene	0.00	0.00	0.00	0.00	7.60	0.00	6.56	0.00
fluoranthene	11.30	10.86	12.73	10.92	12.66	11.64	11.83	11.45
pyrene	4.12	4.54	17.49	13.38	19.32	13.98	16.70	15.86
C-1 fluoranth./pyrenes	3.07	3.23	32.88	25.29	41.62	27.83	79.80	44.36
benz-a-anthr.	0.00	0.97	0.95	1.19	0.00	1.28	0.00	0.00
chrysene	0.00	0.00	20.51	13.33	19.84	12.43	18.72	15.68
C-1 chrysenes	0.00	0.00	10.49	8.63	11.23	7.78	4.41	11.02
C-2 chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C-3 chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C-4 chrysenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
benzo-b-fluoranthene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
benzo-k-fluoranthene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
benzo-e-pyrene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
benzo-a-pyrene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
perylene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
indeno-123-cd-pyrene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
dibenzo-a,h-anthr.	0.00	0.00	13.35	0.00	0.00	0.00	0.00	0.00
benzo-g,h,i-perylene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total PAC	546.54	656.97	5640.03	4172.91	8799.89	6374.36	9635.09	8097.19
	mean	SD	mean	SD	mean	SD	mean	SD
	602	78	4906	1037	7587	1715	8866	1087

Appendix 9. PAC concentration in *Neocalanus* tissue (Chapter 4). Sample ID refers to chain of custody identification (Appendix 1); MDL = method detection limits on a 80% significance level

	sample ID	1200935	1200936	1200937	1200938	1200939
	wet weight (g)	0.0217	0.0246	0.0234	0.0252	0.0224
	matrix	TISSUE	TISSUE	TISSUE	TISSUE	TISSUE
Surrogate Recov (%)						
NAPHTHALENE d-8		86.53	86.37	81.47	82.10	79.69
ACENAPHTHENE d-10		85.55	87.66	85.66	86.28	83.62
PHENANTHRENE d-10		83.54	86.15	85.29	84.09	85.85
CHRYSENE d-12		77.60	82.88	80.95	78.49	85.02
BENZO-a-PYRENE d-12		83.75	85.86	83.51	79.91	86.25
PERYLENE d-12		83.75	87.51	85.28	83.39	88.90
Analyte conc.(ng/g):	MDL, ng					
naphthalene	5.73	0.00	0.00	0.00	0.00	0.00
N1	13.07	0.00	0.00	0.00	0.00	0.00
C-2 naphthalenes	1.90	88.92	119.80	129.61	94.02	181.93
C-3 naphthalenes	2.27	114.23	331.59	310.41	389.02	734.28
C-4 naphthalenes	2.27	0.00	140.48	0.00	143.50	372.67
biphenyl	7.97	0.00	0.00	0.00	0.00	0.00
acenaphthylene	6.40	0.00	0.00	0.00	0.00	0.00
acenaphthene	2.60	180.00	0.00	0.00	0.00	0.00
fluorene	5.90	0.00	0.00	0.00	0.00	0.00
C-1 fluorenes	5.90	0.00	0.00	0.00	0.00	0.00
C-2 fluorenes	5.90	0.00	0.00	0.00	242.83	494.77
C-3 fluorenes	5.90	0.00	0.00	0.00	0.00	0.00
dibenzothiophene	3.80	0.00	0.00	0.00	0.00	188.22
C-1 dibenzothiophenes	3.80	0.00	161.20	0.00	152.26	522.30
C-2 dibenzothiophenes	3.80	0.00	160.52	0.00	151.83	385.98
C-3 dibenzothiophenes	3.80	0.00	0.00	0.00	0.00	0.00
phenanthrene	3.70	178.46	240.07	197.17	239.71	506.29
C-1 phenanthr./anthr	8.43	0.00	0.00	0.00	0.00	1003.84
C-2 phenanthr./anthr	8.43	0.00	0.00	0.00	0.00	766.99
C-3 phenanthr./anthr	8.43	0.00	0.00	0.00	0.00	0.00
C-4 phenanthr./anthr	8.43	0.00	0.00	0.00	0.00	0.00
anthracene	2.97	0.00	0.00	0.00	0.00	0.00
fluoranthene	6.93	0.00	0.00	0.00	0.00	0.00
pyrene	7.13	0.00	0.00	0.00	0.00	0.00
C-1 fluoranth./pyrenes	7.13	0.00	0.00	0.00	0.00	0.00
benz-a-anthracene	2.47	0.00	0.00	0.00	0.00	0.00
chrysene	3.93	0.00	0.00	0.00	0.00	0.00
C-1 chrysenes	3.93	0.00	0.00	0.00	0.00	0.00
C-2 chrysenes	3.93	0.00	0.00	0.00	0.00	0.00
C-3 chrysenes	3.93	0.00	0.00	0.00	0.00	0.00
C-4 chrysenes	3.93	0.00	0.00	0.00	0.00	0.00
benzo-b-fluoranthene	11.27	0.00	0.00	0.00	0.00	0.00
benzo-k-fluoranthene	5.57	0.00	0.00	0.00	0.00	0.00
benzo-e-pyrene	6.63	0.00	0.00	0.00	0.00	0.00
benzo-a-pyrene	5.33	0.00	0.00	0.00	0.00	0.00
perylene	7.50	0.00	0.00	0.00	0.00	0.00
indeno-123-cd-pyrene	3.53	0.00	0.00	0.00	0.00	0.00
dibenzo-a,h-anthracene	4.40	0.00	0.00	0.00	0.00	0.00
benzo-g,h,i-perylene	12.30	0.00	0.00	0.00	0.00	0.00
Total PAC		561.60	1153.66	637.19	1413.17	5157.28

Appendix 9 (cont.).

	sample ID	1200940	1200941	1200942	1200943	1200944
	wet weight (g)	0.0225	0.0193	0.0207	0.0166	0.0241
	matrix	TISSUE	TISSUE	TISSUE	TISSUE	TISSUE
Surrogate Recov. (%):						
NAPHTHALENE d-8		85.00	84.96	80.22	83.17	84.92
ACENAPHTHENE d-10		86.88	85.39	82.19	84.93	87.73
PHENANTHRENE d-10		85.72	84.45	84.88	85.57	88.58
CHRYSENE d-12		81.88	75.52	80.36	81.50	83.48
BENZO-a-PYRENE d-12		84.25	70.83	80.08	83.14	85.72
PERYLENE d-12		86.59	76.45	83.77	85.78	88.30
Analyte conc. (ng/g):	MDL, ng					
naphthalene	5.73	0.00	0.00	0.00	0.00	0.00
N1	13.07	0.00	0.00	0.00	0.00	0.00
C-2 naphthalenes	1.90	149.09	124.27	481.04	375.75	332.39
C-3 naphthalenes	2.27	697.38	618.06	1965.58	1824.52	1556.43
C-4 naphthalenes	2.27	280.62	276.83	668.62	601.69	531.31
biphenyl	7.97	0.00	0.00	0.00	0.00	0.00
acenaphthylene	6.40	0.00	0.00	0.00	0.00	0.00
acenaphthene	2.60	0.00	206.69	153.61	0.00	0.00
fluorene	5.90	0.00	0.00	0.00	0.00	0.00
C-1 fluorenes	5.90	0.00	0.00	704.71	670.08	576.84
C-2 fluorenes	5.90	437.66	433.78	861.45	790.92	681.32
C-3 fluorenes	5.90	0.00	0.00	0.00	0.00	0.00
dibenzothiophene	3.80	0.00	0.00	738.73	671.38	645.26
C-1 dibenzothiophenes	3.80	476.80	409.72	932.55	950.15	811.43
C-2 dibenzothiophenes	3.80	359.59	315.32	550.88	569.64	462.42
C-3 dibenzothiophenes	3.80	0.00	0.00	212.42	0.00	0.00
phenanthrene	3.70	467.28	472.19	1577.62	1472.69	1246.18
C-1 phenanth./anthr.	8.43	885.49	774.41	1794.30	1826.61	1553.58
C-2 phenanth./anthr.	8.43	710.52	660.84	1139.73	1182.43	940.91
C-3 phenanth./anthr.	8.43	0.00	0.00	0.00	0.00	0.00
C-4 phenanth./anthr.	8.43	0.00	0.00	0.00	0.00	0.00
anthracene	2.97	0.00	0.00	0.00	0.00	0.00
fluoranthene	6.93	0.00	0.00	0.00	0.00	0.00
pyrene	7.13	0.00	0.00	0.00	0.00	0.00
C-1 fluoranth./pyrenes	7.13	0.00	0.00	0.00	0.00	0.00
benz-a-anthracene	2.47	0.00	0.00	0.00	0.00	0.00
chrysene	3.93	0.00	0.00	0.00	0.00	0.00
C-1 chrysenes	3.93	0.00	0.00	0.00	0.00	0.00
C-2 chrysenes	3.93	0.00	0.00	0.00	0.00	0.00
C-3 chrysenes	3.93	0.00	0.00	0.00	0.00	0.00
C-4 chrysenes	3.93	0.00	0.00	0.00	0.00	0.00
benzo-b-fluoranthene	11.27	0.00	0.00	0.00	0.00	0.00
benzo-k-fluoranthene	5.57	0.00	0.00	0.00	0.00	0.00
benzo-e-pyrene	6.63	0.00	0.00	0.00	0.00	0.00
benzo-a-pyrene	5.33	0.00	0.00	0.00	0.00	0.00
perylene	7.50	0.00	0.00	0.00	0.00	0.00
indeno-123-cd-pyrene	3.53	0.00	0.00	0.00	0.00	0.00
dibenzo-a,h-anthracene	4.40	0.00	0.00	0.00	0.00	0.00
benzo-g,h,i-perylene	12.30	0.00	0.00	0.00	0.00	0.00
Total PAC		4464.42	4292.10	11781.23	10935.87	9338.08

Appendix 10. UV and light intensities and dose during sunlight exposures (Chapter 4).

RFF = visible light.

Time 051601	RFF (W*m^-2)	time*intensity	
13:44	140.80	4083.2	
14:15	212.10	6363	
14:45	285.40	9989	
15:20	133.90	1339	start
15:30	190.90	1909	
15:40	152.40	1524	
15:50	170.50	1705	
16:00	167.50	1675	
16:10	144.30	1443	
16:20	163.00	1630	
16:30	134.10	1341	
16:40	137.50	1375	
16:50	125.70	1257	
17:00	137.20	1372	
17:10	133.50	1335	
17:20	216.20	2162	
17:30	108.90	1089	
17:40	176.50	1765	
17:50	89.41	894.1	
18:00	59.10	591	
18:10	67.81	678.1	
18:20	53.63	536.3	
18:30	30.17	301.7	
18:40	75.85	758.5	
18:50	26.13	261.3	
19:00	20.76	207.6	
19:10	25.11	251.1	

Time 051701	RFF (W*m^-2)	time*intensity
10:10	172.30	1723
10:20	209.00	2090
10:30	103.20	1032
10:40	128.00	1280
10:50	146.70	1467
11:00	165.00	1650
11:10	129.30	1293
11:20	128.90	1289
11:30	211.80	2118
11:40	131.90	1319
11:50	171.60	1716
12:00	153.20	1532
12:10	321.50	3215
12:20	168.70	1687
12:30	201.70	2017
12:40	159.80	1598
12:50	207.20	2072
13:00	240.90	2409
13:10	205.10	2051
13:20	162.10	1621
13:30	125.80	1258
		36437

integrated RFF dose day 1:	27400.7
integrated RFF dose day 2:	36437
integrated RFF dose total:	63837.7

Time 051601	UVA (W*m^-2)	time*intensity	
13:44	23.82	690.78	
14:15	32.50	975	
14:45	39.87	1395.45	
15:20	25.77	257.7	start
15:30	26.16	261.6	
15:40	23.78	237.8	
15:50	26.24	262.4	
16:00	28.98	289.8	
16:10	25.05	250.5	
16:20	26.74	267.4	
16:30	22.84	228.4	
16:40	23.38	233.8	
16:50	21.03	210.3	

Time 051701	UVA (W*m^-2)	time*intensity
10:10	25.00	250
10:20	30.20	302
10:30	15.80	158
10:40	16.26	162.6
10:50	25.22	252.2
11:00	20.56	205.6
11:10	19.56	195.6
11:20	17.32	173.2
11:30	29.11	291.1
11:40	21.46	214.6
11:50	23.26	232.6
12:00	23.30	233
12:10	42.20	422

Appendix 10 (cont.)

17:00	23.85	238.5
17:10	20.76	207.6
17:20	30.34	303.4
17:30	19.28	192.8
17:40	24.02	240.2
17:50	15.02	150.2
18:00	11.93	119.3
18:10	12.11	121.1
18:20	10.49	104.9
18:30	11.28	112.8
18:40	7.34	73.38
18:50	5.36	53.63
19:00	4.15	41.48
19:10	4.94	49.36

integrated UVA dose day 1:	4508.35
integrated UVA dose day 2:	5096.4
integrated UVA dose total:	9604.75

12:20	28.92	289.2
12:30	20.82	208.2
12:40	24.46	244.6
12:50	27.41	274.1
13:00	27.98	279.8
13:10	27.96	279.6
13:20	22.16	221.6
13:30	20.68	206.8
		5096.4

Time 051601	**UVB (mW*m^-2)**	time*intensity	
13:44	817.80	23716.2	
14:15	1118.00	33540	
14:45	1270.00	44450	
15:20	734.90	7349	start
15:30	749.10	7491	
15:40	689.90	6899	
15:50	758.60	7586	
16:00	695.10	6951	
16:10	674.90	6749	
16:20	697.50	6975	
16:30	634.70	6347	
16:40	624.50	6245	
16:50	577.30	5773	
17:00	618.50	6185	
17:10	497.30	4973	
17:20	614.90	6149	
17:30	435.10	4351	
17:40	496.00	4960	
17:50	339.10	3391	
18:00	290.40	2904	
18:10	290.30	2903	
18:20	248.10	2481	
18:30	172.10	1721	
18:40	141.90	1419	
18:50	119.20	1192	
19:00	86.57	865.7	
19:10	105.12	1051.2	

integrated UVB dose day 1:	112910.9
integrated UVB dose day 2:	138083
integrated UVB dose total:	250993.9

Time 051701	**UVB (mW*m^-2)**	time*intensity
10:10	560.2	5602
10:20	623	6230
10:30	390	3900
10:40	439.5	4395
10:50	512.2	5122
11:00	695.1	6951
11:10	512.1	5121
11:20	486.1	4861
11:30	730.2	7302
11:40	566.4	5664
11:50	643.4	6434
12:00	633.5	6335
12:10	1004	10040
12:20	706.8	7068
12:30	724.4	7244
12:40	677.1	6771
12:50	724.2	7242
13:00	977.8	9778
13:10	835	8350
13:20	718.1	7181
13:30	649.20	6492
		138083

Appendix 10 (cont.).

060501 Time	vis. light (W*m^-2)	time*intensity vis. light	UVA (W*m^-2)	time*intensity UVA	UVB (mW*m^-2)	time*intensity UVB
11:45	158.8	1588	20.8	208	710	7100
11:55	180	1800	26.24	262.4	780.3	7803
12:05	181.7	1817	26.45	264.5	795.5	7955
12:15	202.3	2023	28.86	288.6	857.8	8578
12:25	201.6	2016	30.45	304.5	905.7	9057
12:35	172.5	1725	25.29	252.9	783	7830
12:45	216	2160	31.08	310.8	931.5	9315
12:55	243	2430	35.57	355.7	1117	11170
13:05	241.2	2412	36.19	361.9	1118	11180
13:15	165.6	1656	32.03	320.3	905.8	9058
13:25	150.6	1506	24.04	240.4	774.4	7744
13:35	135.9	1359	21.71	217.1	703.3	7033
13:45	126.6	1266	20.58	205.8	631.8	6318
13:55	119.8	1198	19.21	192.1	595.3	5953
14:05	121.9	1219	19.4	194	590.3	5903
14:15	101.92	1019.2	15.9	159	476	4760
14:25	111.6	1116	17.39	173.9	526.7	5267
14:35	116.2	1162	18.03	180.3	547.5	5475
14:45	181.8	1818	26.48	264.8	785.7	7857
14:55	122.9	1229	20.06	200.6	598.6	5986
15:05	136.6	1366	21.46	214.6	678.6	6786
15:15	164.3	1643	26.52	265.2	789.3	7893
15:25	159.5	1595	25.67	256.7	715.5	7155
15:35	116.6	1166	19.55	195.5	584.3	5843
15:45	117.4	1174	19.51	195.1	606.1	6061
15:55	136.1	1361	20.68	206.8	587.7	5877
16:05	124.5	1245	20.14	201.4	582.7	5827
16:15	137.7	1377	27.9	279	693.5	6935
16:25	136.6	1366	25.53	255.3	852	8520
integrated dose total:		44812.2		7027.2		212239